Biodiversity of wetlands and its implications for pondscape conservation

By

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Declaration

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Summary

Wetlands are socioeconomically important, providing valuable goods and services, and supporting high levels of biodiversity, making them a top conservation priority. KwaZulu-Natal agroforestry plantations have large ecological networks of remnant land, rich in wetlands and ponds, having similar biodiversity value to protected areas. It is important that we continue to make wetlands a priority by developing efficient conservation and land management techniques. More knowledge is required to make recommendations not only to protect water sources but also freshwater biodiversity.

Biological surrogates are useful in conservation planning. However, they need to be easy and cost-effective to sample, show high sensitivity to anthropogenic change, and ideally have similar assemblage patterns to other taxa. Chapter 2 tested aquatic taxa (dragonflies, aquatic beetles and bugs, frogs, and aquatic vegetation) as potential surrogates for wetland-associated communities but found no significant cross-taxon congruence to suggest a single taxon as an indicator. Cross taxon congruence is often due to shared response to environmental gradients, but there were too few similar responses to indicate congruence. The umbrella index was evaluated for wetland conservation, and was successfully applied here, maximising site prioritisation by focusing on both rare and co-occurring umbrella species. Three different taxa were tested with the umbrella index, and although cross-group surrogates showed high co-occurrence, poor cross-taxon correlations show that surrogate species should only be used to represent their own groups. Thus, a multi-taxon approach is required for effective conservation management of wetland sites.

Wetlands have margins that are transitional areas (ecotones) between aquatic and terrestrial environments composed of zones, and where communities overlap. However, little is known about the assemblage patterns of taxa across these ecotones. The manner in which taxa utilise the ecotone has important implications for conservation management. Chapter 3 showed that dragonflies were distributed across the entire ecotone, peaking in the marsh zones where marginal vegetation is optimal for life history functions. Butterflies peaked in the grassland zone, but had marsh-associated species, while grasshoppers had high fidelity with dry grassland. Within each site, species generally moved freely across the zones, demonstrating little species turnover between zones. However, high species turnover occurred among the sites, which has implications at the pondscape (multiple ponds) level.

Pondscapes are networks of smaller ponds or wetland patches, including the terrestrial matrix. Individual ponds are highly heterogeneous due to local fluctuations, and thus pondscapes have higher conservation value. Chapter 2 showed that at least twenty ponds should be conserved to adequately conserve most taxa in a pondscape, indicating the general size of a pondscape. Chapter 3 found that pond ecotones have high species turnover, with each pond supporting a different set of species, and

the pondscape supporting a much larger set of species than individual ponds. Terrestrial species responded to water conditions even though they were in the dryland, indicating that ponds have a much larger footprint that just the extent of the water, making terrestrial buffer zones a requirement for the efficient protection of wetland biodiversity. Pondscapes have high conservation priority, and their conservation must include the transition zones and linkages between ponds.

Opsomming

Vleilande is sosio-ekonomies belangrik, lewer verskeie waardevolle goedere en dienste, en ondersteun hoë vlakke van biodiversiteit, wat maak dat hulle ‘n topprioriteit vir bewaring is. Die agrobosbou-aanplantings in KwaZulu-Natal het groot ekologiese netwerke van oorblywende lande wat ryk aan vleilande en damme is, en wat dieselfde biodiversiteitswaarde het as beskermde gebiede. Dit is belangrik dat ons voortgaan om vleilande 'n prioriteit te maak, deur doeltreffende tegnieke vir bewaring en grondbestuur te ontwikkel. Meer kennis is nodig om beter aanbevelings te maak om waterbronne en varswater biodiversiteit te bewaar.

Biologiese surrogate is nuttig in bewaringsbeplanning. Alhoewel, hulle moet maklik en kostedoeltreffend wees om te vind, hoë sensitiwiteit wys teen menslike veranderinge, en ideaal gesproke soortgelyke samestellingspatrone hê as ander taksa. Hoofstuf 2 het akwatiese taksa (naaldekokers, akwatiese kewers en goggas, paddas en waterplantegroei) getoets as potensiële surrogate vir vleilandgeassosieerde gemenskappe, maar het geen beduidende kruistakson kongruensie gevind om 'n enkele takson as 'n aanwyser aan te dui nie. Kruistakson kongruensie is dikwels die gevolg van ‘n gedeelde reaksie teen ‘n omgewingsgradiënte, maar daar was te min soortgelyke reaksies hier om kongruensie aan te dui. Die sambreelindeks was vir die bewaring van vleilande geëvalueer, en was suksesvol hier toegepas, wat die prioritisering van gebiede maksimeer, deur op skaars en saamkomende sambreel spesies te fokus. Drie verskillende taksas is met die sambreelindeks getoets, en alhoewel surrogate tussen groepe hoë mede-voorkoms getoon het, was daar swak korrelasies tussen takse wat toon dat surrogaate slegs gebruik moet word om hul eie groepe voor te stel. Dus is dit nodig om ‘n multi-takson benadering te gebruik vir effektiewe bewaringsbestuur van vleilande.

Vleilande het kante wat oorgangsgebiede (ekotone) is tussen water- en landomgewings, en betstaan uit sones waar gemeenskappe oorvleuel. Daar is egter min bekend oor die samestellingspatrone van taksa oor hierdie ekotone. Die manier hoe taksas die ekotone gebruik het belangrike implikasies vir bewaringsbestuur. Hoofstuk 3 het getoon dat naaldekokers oor die hele ekotoon versprei is, met ‘n piek in die moerasgebiede waar marginale plantegroei optimaal is vir naaldekoker lewens funksies. Skoenlappers het 'n hoogtepunt bereik in die grasveldsone, maar daar was skoenlappers wat verband was met die moerasgebied, terwyl sprinkane ‘n hoë getrouheid aan droë grasveld getoon het. Binne elke gebied het spesies, oor die algemeen, vrylik oor die sones beweeg, wat lae spesie-omset tussen sones toon. Alhoewel, daar was ‘n hoë spesie-omset tussen gebiede wat dus implikasies het vir die dambeeld (klomp damme) in die landskap.

Dambeelde is netwerke van kleiner damme of vleilande, insluitend die landmatriks. Individuele damme is hoogs heterogeen as gevolg van plaaslike skommelinge, en daarom het dambeelde hoër bewaringswaarde. Hoofstuk 2 het gewys dat ten minste twintig damme bewaar moet word om die meeste taksas in 'n dambeeld te bewaar, wat die algemene grootte van ‘n dambeeld aandui. Hoofstuk 3 het gevind dat die ekotone van damme ‘n hoë spesie-omset het, met dat elke dam 'n ander stel spesies ondersteun, wat maak dat die dambeeld 'n groter hoeveelheid spesies ondersteun as individuele damme. Aardse spesies het op die watertoestande gereageer, alhoewel hulle in die droëland voorgekom het, wat daarop aandui dat damme 'n baie groter voetspoor het as net die omvang van die water, wat landelike buffergebiede 'n vereiste maak vir doeltreffende beskerming van vleiland biodiversiteit. Dambeelde het ‘n hoë bewaringsprioriteit en die bewaring daarvan moet die oorgangsones en skakels tussen damme insluit.

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Chapter 1: Introduction

1.1 Wetland functions and threats

Freshwater systems are among the most threatened ecosystems, with higher rates of extinction than terrestrial or marine ecosystems (Sala et al. 2000; Millennium Ecosystem Assessment 2005; Dudgeon et al. 2006; Kingsford et al. 2016). According to the Ramsar Convention, wetlands are defined as "areas of marsh, fen, peatland or water, whether natural or artificial, permanent or temporary, with water that is static or flowing, fresh, brackish or salt" (Ramsar Convention 1994). They are among the world's most productive environments (Sandham et al. 2008) covering > 6% of the Earth's surface (Cools et al. 2013) and provide valuable goods and services to society such as provisioning, regulating, cultural and supporting services that produce direct or indirect economic value (Barbier 1993; Millennium Ecosystem Assessment 2005).

Wetlands are often referred to as environmental 'kidneys' due to the functions they perform, including groundwater replenishment, water purification, sediment and nutrient retention and flood control (Sandham et al. 2008; Oberholster et al. 2014; Kyerematen et al. 2018). They have also been called biological 'supermarkets', as they support extensive food webs and rich biodiversity including aquatic, semiaquatic, and terrestrial fauna and flora (Sandham et al. 2003; Belle et al. 2018). Despite legislation designed to protect them, wetlands are among the most threatened ecosystems in the world and degrade at a higher rate than other ecosystems (Turner et al. 2000; Millennium Ecosystem Assessment 2005). Wetland functions are insufficiently appreciated, and there tends to be a lack of understanding of their ecological and socioeconomic importance, leading to policy and land management deficiencies (Terer et al. 2004). The multiple benefits provided by wetlands means that there are competing wetland-use priorities, thus management practices must balance this, and the threats of degradation from external pressures such as climate change and urban development (Cools et al. 2013).

Freshwater habitats are especially vulnerable to human activities and environmental change because they are highly sought after for urban and agricultural water supply. The major threats to freshwater biodiversity are overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasion by exotic species, exacerbated further by global-scale environmental change (Allan and Flecker 1993; Jackson et al. 2001; Malmqvist and Rundle 2002; Revenga et al. 2005; Dudgeon et al. 2006). Trade-offs between conservation of freshwater biodiversity and human consumption of ecosystem goods and services are necessary. Freshwater biodiversity conservation is

challenging as it requires control over the upstream/downstream reaches, riparian zones, and the surrounding land to be effective (Dudgeon et al. 2006).

In South Africa, wetlands have been identified as the most threatened type of ecosystem, driven by increasing land and water demands, with estimations of 30-90% of wetlands lost or severely degraded due to agriculture and poor land management depending on the region (Kotze et al. 1995; Lannas and Turpie 2009; Cools et al. 2013; Dini and Bahadur 2016). According to Driver et al (2012), South African wetlands cover just 2.4% of the country's surface area due to climate conditions not being conducive to the persistence of surface water and average evaporation exceeding rainfall (Dini and Everard et al. 2016). Higher levels of threat in freshwater taxa have been reported in South Africa than the rest of the southern African region as South African urban areas are expanding faster than other African countries (Darwall et al. 2009). This means that stemming the loss and degradation of South African wetlands is essential, and without which, there will be severe consequences, considering the semiarid context of the country (Kotze 1995; Dini and Everard et al. 2016). For example, wetlands manage water retention during flooding which is crucial in a dry country like South Africa as it helps to prevent soil erosion (Phethi and Gumbo 2019). The loss and degradation of South African wetlands greatly contributes to our growing water scarcity problems.

1.2 Importance of a pondscape

Conservation and management of freshwater ecosystems often focuses on running water and larger water bodies, overlooking relatively smaller waterbodies, such as ponds (Oertli et al. 2009). There is growing realisation that these small wetland patches (1 m^2 – 5 ha) are of important conservation value, hosting high and unique biodiversity, and provide a variety of socioeconomic services. However, most ponds or wetlands receive little protection because they are small in comparison to rivers and lakes (Gibbons 2003). Ponds should never be considered in isolation, but rather the pond landscape or 'pondscape' should be considered in its entirety (Oertli et al. 2009; Rannap et al. 2009; Triest and Sierens 2009). A pondscape can be defined as a network of ponds and their surrounding terrestrial matrix (Hill et al. 2018). Pondscapes contribute more to aquatic biodiversity than bigger freshwater bodies such as lakes and rivers (Davies et al. 2008), and provide essential habitat for rare and threatened species, while also functioning as refuges in urban and agricultural landscapes (Chester and Robson 2013).

A pondscape, or network of smaller ponds, has higher taxonomic richness and conservation value than one large pond (Oertli et al. 2002). In UK urban ponds, higher pond density is associated with higher species richness (Gledhill et al. 2008). Hill et al. (2018) suggests that a network of smaller freshwater habitats (pondscapes) must be incorporated alongside large-scale habitat networks to

improve the effectiveness of landscape conservation. For example, in South African agroforestry, large ecological networks of remnant land, set aside to mitigate the effects of agroforestry, are rich in natural and artificial pondscapes and have similar biodiversity value to those in protected neighbouring areas (Pryke et al. 2015). Urban and agricultural landscapes have barriers such as roads and planted areas, limiting the dispersal and colonisation of pond biota, thus pondscape management should aim to increase connectivity between ponds (Hill et al. 2018).

1.3 Agroforestry and ecological networks

Plantation forestry is known to have negative impacts on biodiversity (Lindenmayer et al. 2003; Eckehard et al. 2008) with many researchers finding low levels of biodiversity in plantation compartments, especially when non-native trees are used (Pryke and Samways 2009; Bremer and Farley 2010). There is vigorous debate on the implications for conservation of biodiversity in plantation forests, as it has been suggested that plantations may play an important role in biodiversity conservation and restoration of forest species (Hartley 2002; Carnus et al. 2006; Brockerhoff et al. 2008) when management practices can balance environmental and economic goals (Bremer and Farley 2010).

Within South Africa, KwaZulu-Natal is one of the two provinces suitable for forestry, and lies within threatened grassland and indigenous forest biomes (Neke and du Plessis 2004; Samways et al. 2010). KwaZulu-Natal also falls within the Maputaland-Pondoland-Albany hotspot, recognised as an important region of plant endemism containing high natural grassland biodiversity (Lipsey and Hockey 2010), and is also naturally subject to El Niño events, causing periodic drought and flooding (Samways et al. 2010). However, increasing human population density is causing the region to become impacted by anthropogenic activities, and is considered one of the most degraded biomes in the world (Neke and du Plessis 2004; Bailey et al. 2015).

The timber industry occupies 1.5% of the country's surface area (DWAF 2006), with an average of one-third of a plantation left unplanted to maintain and conserve interconnected corridors and patches containing natural grassland, wetlands, streams, ponds, and indigenous forest (Samways et al. 2010; Samways and Pryke 2016). These unplanted areas form ecological networks (ENs), strips of remnant habitat consisting of natural patches and corridors connecting them, with the aim to conserve biodiversity (Jongman 1995). ENs are essential for the maintenance of habitat quality and heterogeneity and are an effective extension of protected areas by supporting complementary arthropod assemblages (Kietzka et al. 2015; Samways and Pryke 2016; van Schalkwyk et al. 2017). The management of ENs ensures improved connectivity between habitat patches (Pryke and Samways 2016), with larger corridors ensuring a larger amount of core habitat is conserved and

smaller corridors facilitating movement across the production landscape (Pyke and Samways 2001; Samways and Pryke 2016; van Schalkwyk et al. 2017). ENs are considered to be as successful as adjacent protected areas in conserving biodiversity, with previous studies highlighting their effectiveness in the conservation of terrestrial taxa (Bazelet and Samways 2011; Gaigher et al. 2019; Joubert-van der Merwe et al. 2019) and aquatic taxa (Pryke et al. 2015; Kietzka et al. 2015; Briggs et al. 2019).

1.4 The significance of insects, frogs, and aquatic plants

Insects are the most diverse group of animals on Earth, occupying almost every microhabitat except the oceanic benthic zone and the ice-covered poles (Grimaldi and Engel 2005; Crespo-Pérez et al. 2020) with arthropods constituting 81.3% of described animal species excluding Protista (Samways 1993). Insects are responsible for many ecosystem services, including seed dispersal, pollination, organic matter processing, nutrient cycling, and water filtration (Hoehn et al. 2008; Jouquet et al. 2011; Footit and Adler 2017; Jankielsohn 2018). They contribute to the regulation of plant diversity, photosynthesis, soil fertility, ecosystem structure and water quality, providing a significant contribution to conservation (Crespo-Pérez et al. 2020). Insects are highly sensitive to environmental change, and many are threatened by agriculture, urbanisation, and global climate change.

Aquatic insects spend one or more life stage in the water, with the majority living in the water as immatures and progressing to terrestrial habitats as adults. They contribute to 6% of known insect species, accounting for 80% of aquatic animal diversity (Dijkstra et al. 2014). Their reliance on multiple habitat types makes them particularly vulnerable to environmental change and anthropogenic activities such as poor water management, pollution, and the disruption of river courses (Darwall et al. 2012). Aquatic insects are also subject to indirect pressures from the terrestrial component of their habitat, such as vegetation removal, invasion by exotic species, erosion, and urbanisation (Revenga et al. 2005).

Amphibians comprise a unique component of vertebrate fauna with over 7 000 described species globally. Amphibians are found in virtually all terrestrial and freshwater habitats, living almost everywhere except in extremely cold and extremely dry regions and many oceanic islands (Frost et al. 2006). Approximately 88% of amphibian species are classified in the order Anura, frogs and toads. Anuran lifestyles vary between species, some being purely aquatic, aquatic and terrestrial, terrestrial, arboreal, and fossorial (Crump 2009). Whilst frogs have evolved diverse life histories, majority have an aquatic larval (tadpole) stage, which means they are dependent on multiple habitat types to complete their life histories. Anuran larvae are most often herbivorous, regulating aquatic flora whilst adult anurans are most often insectivorous, with their foraging activities playing an important role in

regulating invertebrate populations (Wells 2007). Males of most species emit a species-specific call to attract females for mating (Crump 2009). Anuran complex life histories and thin, permeable skin makes them highly susceptible to environmental stress and are currently undergoing a global-scale population decline due to a variety of anthropogenic factors, with the most critical being habitat destruction and fragmentation (Frost et al. 2006).

Angiosperms are the largest and most diverse group within the kingdom Plantae and represent approximately 80% of all known plants. Aquatic angiosperms have adapted to living in water-saturated soils of aquatic environments (freshwater or saltwater) and are often referred to as hydrophytes or macrophytes (Brix 1994). Macrophytes are key components of aquatic and wetland ecosystems as they are primary producers, and their stems and roots provide shelter, and emergence and perching sites for invertebrates (Rejamankova 2011; Kietzka et al. 2015). Macrophytes are vital in healthy wetland systems as they directly influence nutrient cycling, they remove excess nutrients from the water, promote sedimentation, impede erosion, and provide habitat heterogeneity for invertebrates and other wetland associated groups (Brix 1997; Thomaz et al. 2008; Horppila et al. 2013).

1.5 Using bioindicators for wetlands

The monitoring of wetland biodiversity is vital in conservation planning. However, surveying wetland biodiversity is often accompanied by financial constraints, time limitations and expertise requirements (Guareschi et al. 2015). For efficient biomonitoring, the use of surrogate taxa to represent the community may relieve some of these constraints and has the potential to simplify data requirements for conservation (Leal et al. 2010), reducing cost requirements and enabling prioritization of conservation efforts despite limited time (Bried et al. 2007). This is because relying on a small number of species as proxies for the protection of the whole community allows for focussed conservation effort (Bried et al. 2007).

Biological indicators are species or groups of species that reflect information on an ecological system with the purpose of indicating ecosystem integrity (Hunter et al. 2016). The use of biological indicators for assessing biodiversity of a system has received an increasing amount of interest in conservation planning (Leal et al. 2010). A good indicator is sensitive to environmental change, responds to environmental stressors in a predictable way, shows similar biodiversity patterns to other groups, is taxonomically and ecologically understood, and is easy and cost effective to monitor (Dale and Beyeler 2001; Rooney and Bayley 2012; Guareschi et al. 2015). The reliance on a single surrogate assemblage is recommended only when congruency patterns persist across the human disturbance gradient, especially as previous studies have shown human disturbance to decrease congruency

between assemblages (Rooney and Bayley 2012). A multi-taxon approach is best when considering surrogates because the inclusion of many indicator species will improve the potential of finding a suite of surrogates that most efficiently conserves the ecosystem.

Aquatic insects are among the most frequently used groups in the biological assessment of water quality worldwide. Aquatic beetles are commonly suggested as an indicator for aquatic or riparian communities (Bilton 2006; Gioria et al. 2010; Briggs et al. 2019) and are the most speciose and abundant macroinvertebrate group. Aquatic beetles are components of SASS (South African Scoring System), a widely used, rapid biomonitoring technique that assesses freshwater ecosystem quality using the presence of benthic macroinvertebrates based on family level identification and is applicable only to lotic systems (Dickens & Graham 2002). Aquatic bugs, although less speciose, are diverse in their functions and also form components of SASS. The larval stages of dragonflies are also components of SASS.

The Dragonfly Biotic Index (DBI) uses the presence of adult dragonfly species as a measurement of freshwater habitat conditions (Simaika and Samways 2009; Samways and Simaika 2016). Dragonflies are an ideal indicator group because they are amphibiotic and represent the aquatic-terrestrial interface, with an aquatic larval stage directly dependent on water conditions and a highly mobile terrestrial adult stage that can easily move to more suitable environments. Adult dragonflies also demonstrate a rapid response to marginal vegetation (d'Amico et al. 2004; Golfieri et al. 2016; Valente-Neto et al. 2016) making them good indicators of riparian systems. The DBI is a relatively quick method of scoring the ecological integrity of a freshwater body because adult male dragonflies are colourful and easy to identify in the field (Clark & Samways 1996) and is applicable to a wide variety of both lotic and lentic ecosystems (Samways and Simaika 2016). The DBI has been used effectively in assessing ecosystem integrity, site prioritisation for conservation and monitoring of restoration projects (Simaika and Samways 2011; Samways and Taylor 2004; Magoba and Samways 2010).

Frogs are suggested as a valuable surrogate taxon for invertebrates associated with wetland habitats because they are easy to sample using non-invasive ecoacoustic methods (Kirkman et al. 2012). Ecoacoustics aims to characterise a community using its soundscape. Technological advancement allows for the use of automated recording systems to record species-specific vocalisations over relatively large spatiotemporal scales (Xie et al. 2016). In frogs, mating calls are commonly used as diagnosable characteristics in taxonomy, because these calls are simple, repetitive and are emitted more regularly, thus easily recorded, and analysed (Xie et al. 2016; 2018; Köhler et al. 2017).

Ecoacoustics have the potential to collect a large amount of data over a large spatiotemporal scale and could be a viable method to assess wetlands using frogs as an indicator taxon.

1.6 How big is a wetland?

The terrestrial habitats surrounding wetlands are critical for the management of natural resources because the aquatic-terrestrial interface protects these systems by filtering pollutants and moderating temperature (Semlitsch and Jensen 2001). This terrestrial habitat is essential to the maintenance of wetland biodiversity and the integrity of wetland ecosystems (Harper et al. 2008). Smaller buffers are adequate when the buffer is in good condition, the wetland is of low functional value and the adjacent land has low impact potential, whereas large buffers are necessary for high value wetlands with intense adjacent land use (Castelle et al. 1994). It is generally agreed that a terrestrial buffer zone of 30-60 m is adequate for protection of wetland quality although many studies found this to be inadequate for semiaquatic and terrestrial ecotone species such as numerous species of frogs, salamanders, turtles, and other taxa (Semlitsch and Jensen 2001; Semlitsch and Bodie 2003; Roe and Georges 2007; Harper et al. 2008). Terrestrial habitat for maintaining viable populations of wetland taxa is not the same as the buffer zone required to protect water resources thus, to maintain biodiversity and protect water resources, likely a much larger buffer zone is required.

1.7 Thesis outline and aims

Although wetlands are of high conservation priority, there is still limited knowledge on their biodiversity in comparison to terrestrial systems. The purpose of this study is to investigate the term ‘pondscape’ in terms of its biodiversity and size to inform conservation management of wetlands and more specifically, to gain an understanding of invertebrate and amphibian biodiversity of wetlands within ecological networks of South African agroforestry plantations and contribute to conservation management through wetland bioindicators. Aquatic insects (Bilton 2006; Briggs et al. 2019), dragonflies (Samways and Simaika 2016), frogs (Kirkman et al. 2012), and grasshoppers and butterflies (Bazelet and Samways 2012) have all been previously recommended as indicators and were all investigated for this study. There is limited knowledge on how species assemblages change across the wetland ecotone, which has important implications about the size of a wetland and wetland conservation and management.

The use of a surrogate taxon to represent the community may relieve some financial, temporal, and sampling constraints, and can simplify data requirements for conservation management (Leal et al. 2010), where relying on a few species of the community allows for focussed conservation effort (Bried et al. 2007). In chapter 2, I aim to identify a surrogate or suite of surrogates that can represent

wetlands and wetland associated species assemblages for the efficient conservation of pondscapes. To find a surrogate, I will test the cross-taxon congruence between focal taxa (dragonfly adults and larvae, aquatic beetles and bugs, frogs, and aquatic vegetation), whether any species are potential umbrella taxa and whether they have similar responses to the same environmental gradient. There is higher value in conserving many small ponds and their surrounding terrestrial matrices than one big pond (Hill et al. 2018), and I aim to investigate how many ponds should be conserved to effectively conserve wetland assemblages in a pondscape. I hypothesise that there will be cross-taxon congruence between aquatic and riparian communities due to similar life histories, and that there will be similar responses to environmental variables between groups. Furthermore, I hypothesise that the umbrella index will be successful in the surrogate species approach and that easy to sample frogs can be used as an efficient surrogate for wetland-associated species assemblages in a pondscape.

Wetland ecotones are transition zones occurring where the environmental limits of species and communities overlap and are driven by many factors, which need to be understood for informing conservation and management practices (Brownstein et al. 2015). In chapter 3, I investigate how species assemblage patterns change over the aquatic-terrestrial ecotone by assessing the change in species richness and assemblage composition per zone (open water, deep marsh, shallow marsh, wet meadow, and dry grassland) and beta diversity, including its components, species nestedness and turnover. I determine the drivers of these species assemblage patterns by identifying wet-associated and dry-associated species and assessing their responses to environmental variables. I also investigate the notion that the conservation of many smaller ponds is of greater value than the conservation of one large pond (Oertli et al. 2002; Hill et al. 2018) by assessing each zone at the pondscape level. Using beta diversity and its components, nestedness and turnover, I assess whether species assemblages are consistent across the pondscape or whether each pond has its own set of species per zone. I hypothesise that there will be higher species turnover in the intermediate zones (deep marsh, shallow marsh, and wet meadow) due to overlapping species preferences driven largely by vegetation composition and structure. Furthermore, I hypothesise that each pond will have its own set of species representing vegetation zones due to microhabitat preferences and that wet-associated and dry-associated species will have different environmental drivers, being at opposite extremes of the ecotone.

I conclude with chapter 4, where I discuss the contributions of this research to wetland conservation management and outline management options to promote adequate wetland quality by adequately managing and protecting the pondscape.

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Chapter 2: Degree of cross-taxon congruence between macroinvertebrates and riparian communities to provide recommendations for pondscape size

Abstract

The use of biological surrogates in conservation planning is valuable for conservation prioritisation. Good surrogates ideally are easy and cost-effective to sample, sensitive to anthropogenic change and show similar assemblage patterns to other taxa. Here I tested dragonflies, aquatic insects, frogs, and aquatic plants as potential surrogates for wetland-associated communities, as they are all responsive to changes in the aquatic environment. Some significant cross-taxon correlations were found but were too weak to consider potential surrogacy due to too few similar responses to the same environmental gradients. The performance of the umbrella index was evaluated for wetland conservation and applied to dragonflies, aquatic insects (beetles, bugs, and dragonfly larvae) and frogs. Aquatic insects were scored at family level due to lack of species-level information, while dragonfly adults and frogs were scored at the species level. The umbrella index identified five dragonfly species, nine aquatic insect species and three frog species as potential umbrella groups. Although all surrogates strongly co-occurred with the other groups, poor cross-taxon correlations show that surrogate species should only be used to represent their own groups in wetlands. The umbrella index functioned well for same-group surrogates and appeared to be flexible, favouring both co-occurring, less sensitive species, and highly sensitive, rarer species, allowing for efficient site prioritisation even in the absence of endemic species. All three endemic species sampled here were identified as surrogate species, with two of them ranking at the top of their group. These results emphasise the need for a multi-taxon approach in conservation management of wetland sites. The pondscape provides a mosaic of ponds that have high heterogeneity due to fluctuations in local conditions. Here I show that we need to conserve at least twenty ponds to conserve most taxa in a pondscape.

Keywords: aquatic insects, dragonflies, frogs, concordance, pond conservation

2.1 Introduction

Cross-taxon congruence refers to the significance and the strength of correlation in assemblage-level biodiversity measures between taxonomic groups across a set of localities (Heino 2010). Strong cross-taxon congruence should yield positive and strong correlations to be useful in conservation planning, meaning that surrogate taxa must show similar assemblage patterns to other taxa at their localities (Heino 2010; Bazelet and Samways 2012). Congruence between different taxonomic groups may occur due to biotic interactions, similar responses to the same environmental variables, and dispersal limitations (Valente-Neto 2018). Ascertaining cross-taxon congruence among potential surrogates

should be performed at the same spatial scale, site size, and latitude for consistency (Westgate et al. 2017). Previous studies have found cross-taxon congruency to be scale-dependent, where studies performed at smaller spatial scales showed weaker patterns of cross-taxon congruence (Padial 2012).

The umbrella species concept is a biodiversity surrogacy method aiming to conserve a large proportion of species in an ecosystem by focusing conservation practices on a single species or a small group of co-occurring species (Fleishman et al. 2000; Bried et al. 2007; Kietzka et al. 2019). The umbrella species concept differs from biodiversity indicators, by focusing not only on congruent patterns of species richness, but also on patterns of co-occurrence, rarity, and sensitivity, and can prioritise sites that require conservation management (Fleishman et al. 2000; Kietzka et al. 2019). The umbrella index was developed as a quantitative method of selecting the best surrogate species or suite of surrogate species to represent other species assemblages. The index consists of three sub-indices, focusing on co-occurrence with other species, occurrence rate, and sensitivity to anthropogenic disturbance (Fleishman et al. 2000; 2001; Bried et al. 2007). Studies demonstrating the application of the umbrella index are limited and has only been done with birds and butterflies (Betrus et al. 2005), dragonflies and wetland plants (Bried et al. 2007), and dragonflies and EPT (Ephemeroptera, Plecoptera and Trichoptera) taxa in rivers (Kietzka et al. 2019).

Odonata are a well-studied order, and unlike other freshwater invertebrate taxa, there is much literature available, particularly on the adult stages, making them taxonomically well understood (Samways 2008; Tarboton and Tarboton 2015). Odonata are widely used indicators of water quality, and their success as indicators has led to the development of the Dragonfly Biotic Index (DBI) (Samways and Simaika 2016). Adult dragonflies are ideal potential surrogates as they are easy to sample and their conspicuous, aesthetic bodies make them easy to identify in the field (Samways and Simaika 2016). The DBI, based principally on the presence of adult species, is mainly used for water quality assessments but has also been used to measure habitat recovery and in site selection for conservation (Simaika and Samways 2012). Dragonflies are considered a good indicator taxon, as they represent the aquatic-terrestrial interface with an aquatic larval stage and a terrestrial adult stage. Adult dragonflies may not respond directly to water conditions, but their larvae remain in the water for weeks prior to emergence, thus water quality conditions must be optimal for successful emergence (Samways and Simaika 2016). In contrast, adults show a rapid response to a change in marginal vegetation when in their terrestrial aerial stage (d'Amico et al. 2004; Golfieri et al. 2016; Valente-Neto et al. 2016).

Aquatic beetles and bugs are gaining attention as water quality indicators as they are widely distributed, occupying almost any aquatic habitat, and are species-rich and diverse in comparison

with many other aquatic insects (Bilton et al. 2006; Benetti and Garrido 2010; Bird et al. 2019; Briggs et al. 2019a). Their families form part of SASS (South African Scoring System), a widely used monitoring technique that assesses river health using the presence of benthic macroinvertebrates (Dickens and Graham 2002). Aquatic beetles are one of the most abundant and speciose macroinvertebrate groups (Bird et al. 2019) with 24 aquatic beetle families (Griffiths et al. 2015) and 17 families of aquatic bugs (Reavell 2003) in South Africa. Aquatic beetles and bugs have adaptations to various ecological conditions and fulfil a range of roles in aquatic systems as they are mostly generalist predators with broad habitat preferences and varying responses to change in vegetation structure and physio-chemical water quality (Bird et al. 2019; Deacon et al. 2018).

Their broad habitat tolerances and high dispersal abilities may suggest that they are poor indicators of anthropogenic effects (Bird et al. 2013, 2019), however, their taxonomy and distribution are not well known which has restricted their potential as surrogates (Reavell 2003; Stals 2003). Previous studies have suggested water beetles as suitable surrogates for aquatic or riparian communities (Bilton 2006; Bloechl et al. 2010; Gioria et al. 2010; Briggs et al. 2019a). Rare and endemic species are often associated with high water quality. Some families, such as Hydraenidae and Elmidae, are sensitive to pollution, having higher sensitivity scores (Dickens and Graham 2002; Benetti and Garrido 2010), and some families are more tolerant to pollution attaining lower sensitivity scores with the SASS method. Their high dispersal ability correlates with that of adult dragonfly dispersal ability, suggesting that we would expect cross-taxon congruence between dragonflies and aquatic beetles and bugs as found in previous studies (Smith et al. 2007; Magoba and Samways 2010).

Frogs have been suggested as a potentially valuable surrogate taxon for invertebrates associated with wetland habitats (Kirkman et al. 2012). Frogs have the potential to be a good surrogate taxon because they are easy to sample in a non-invasive manner by recording the species-specific mating calls during breeding season. Calls can yield species identification and presence or absence of a particular species. Advances in technology permits the use of automated recording systems to record frog vocalisations over relatively large spatiotemporal scales (Dorcas et al. 2009; Xie et al. 2016). Mating calls are commonly used as diagnosable characteristics in taxonomy because these calls are simple, repetitive, and are emitted more regularly, thus are easily recorded and analysed (Xie et al. 2016, 2018; Köhler et al. 2017). Due to their thin, semi-permeable skin, complex life histories, and specific habitat requirements, frogs are highly sensitive to environmental stress (Crump 2009; Simon et al. 2011). Like dragonflies, frogs are amphibiotic, and have an aquatic tadpole stage and an adult terrestrial stage, thus different life stages are likely susceptible to different stressors which provides a complete overview of a wetland. Tadpoles are influential in organic matter dynamics, primary production patterns and algal assemblage structure where adults, being an important trophic link, control

invertebrate populations (Crump 2009). Southern Africa has about 171 known frog species in 33 genera and 13 families (du Preez and Carruthers 2009). Amphibians are one of the most threatened vertebrate groups, with 30% of South African frog species red-listed as threatened (IUCN 2020). It is possible that threatened species can represent a wide range of other species, as they often prefer higher quality habitats. Congruency between invertebrates and amphibians has rarely been studied (Kirkman et al. 2012; Guareschi et al. 2015).

Aquatic plants have also previously been investigated as a potential surrogate of wetland communities (Kirkman et al. 2012; Briggs et al. 2019a), as most wetland-associated taxa are reliant on vegetation for food or microhabitat preferences. Vegetation provides shelter and refuge from predation, food, and emergence and perching sites (Gioria et al. 2010; Kietzka et al. 2015; Briggs et al. 2019a). Alien plant species tend to reduce the diversity of aquatic invertebrates and are one of the major threats to dragonflies in South Africa (Samways and Taylor 2004; Samways and Sharratt 2010; Briggs et al. 2019a). Due to reliance on vegetation structure and composition by most wetland associated species; it is expected that aquatic plants have the potential to represent wetland associated species assemblages.

While there have been several studies investigating cross-taxon congruency between aquatic and riparian communities, there is still uncertainty in the use of surrogates or umbrella species in conservation planning. Some studies have highlighted strong cross-taxon congruence between important aquatic and riparian communities (Smith et al. 2007; Magoba and Samways 2010; Mendes et al. 2017; Briggs et al. 2019a) and others have found weak cross-taxon congruence between aquatic and riparian communities (Heino 2010; Kirkman et al. 2011; Rooney and Bayley 2012; Valente-Neto et al. 2018; de Morais et al. 2018; Carneiro et al. 2019). There is little consistency in the quantitative measurement used in cross-taxon correlations and it is debated whether species richness of a group is a reliable representation of species richness of other groups combined and should be used with caution. Measures of species composition and turnover have been more reliable in finding concordant patterns between taxonomic groups (de Morais et al. 2018).

Conserving whole pondscapes may be a better approach compared with focusing on surrogates. Pondscapes refer to a network of ponds that includes their surrounding terrestrial matrix (Hill et al. 2018). Pondscapes are considered to be complex, biodiverse, and ecologically important freshwater habitats with high habitat heterogeneity and significant conservation value, as they provide refuge habitats for aquatic communities and support semiaquatic and terrestrial fauna and flora (Oertli et al. 2002; Hill et al. 2018; Briggs et al. 2019b). A set of smaller ponds has higher conservation value than one large pond (Oertli et al. 2002), which means conservation management of pondscapes would

encourage connectivity between ponds and encompass a variety of habitat types for many species to complete their life histories (Hill et al. 2018).

This chapter aims to identify a surrogate or suite of surrogates that can represent wetlands and wetland-associated species assemblages for efficient conservation planning of a pondscape. This is based on the following objectives: 1) Testing cross-taxon congruence between the focal taxa (dragonfly adults and larvae, aquatic beetles and bugs, frogs, and aquatic vegetation). 2) Testing for similarity in responses to the same environmental gradients. 3) Evaluating the performance of the umbrella index as a surrogate species approach. 4) Determining the number of ponds to be conserved to effectively conserve of wetland associated species assemblages in a pondscape. I hypothesise that 1) there will be cross-taxon congruence between aquatic and riparian communities due to similar life histories, and 2) that there will be similar responses to environmental variables between groups. Furthermore, I hypothesise that 3) the umbrella index will be successful in the surrogate species approach and that easy to sample frogs can be used as an efficient surrogate for wetland-associated species assemblages in a pondscape.

2.2 Materials and Methods

2.2.1 Study area

Study sites were chosen from five timber plantation mosaics in the KwaZulu-Natal Midlands. These estates are Faber's Hill, Mount Shannon, Good Hope, Linwood, and Mount Gilboa (Figure 2.1). All study sites were in conservation sectors of the estates and were more than 300 m apart and had pine (*Pinus* spp.), eucalyptus (*Eucalyptus* spp.), or natural forest on their borders. I selected 22 sites that had a variety of apparent wetland quality with some being known habitats of the highly endemic Long-Toed Tree frog (*Leptopelis xenodactylus*) (J. Harvey pers. comm.). Quality of sites was based on hydrophilic plant species presence and characteristics of the water body. The ponds were either in open grassland plains or valley bottoms. Fieldwork was conducted over two seasons: early summer (between November and December 2019) and late summer (March 2020).

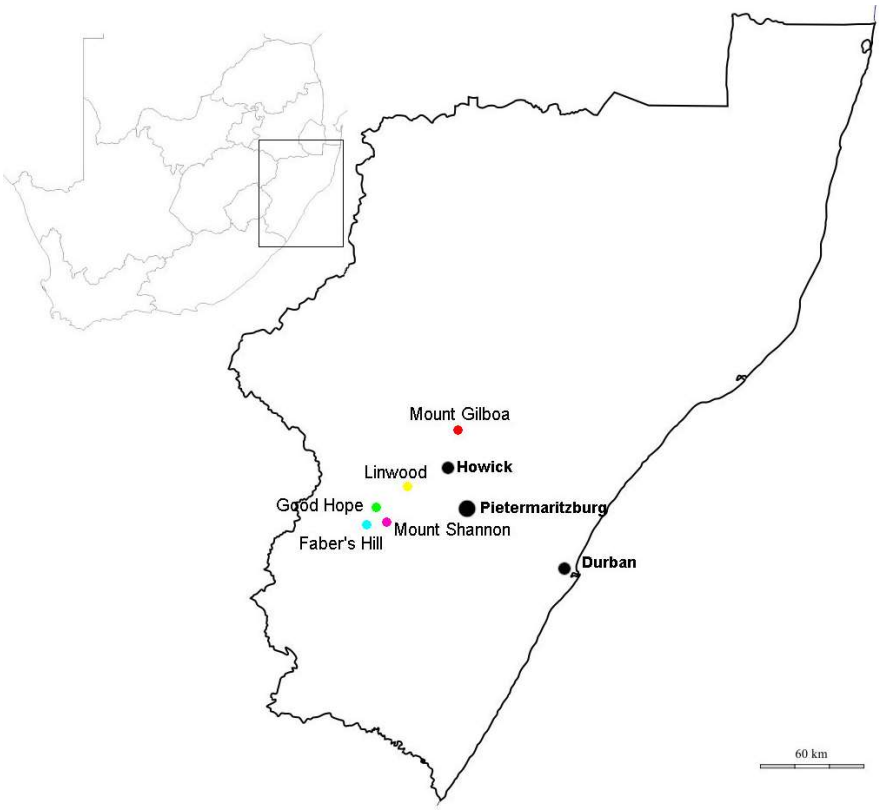

Figure 2.1. Locations of estates, Faber's Hill (blue), Good Hope (green), Mount Shannon (pink), Linwood (yellow) and Mount Gilboa (red). Nearby towns are in bold.

2.2.2 Environmental variables

Categorical variables were recorded at the site level to distinguish differences between the types of ponds sampled: soil type (sand or clay) and waterbody shape (pond waterbody, streamlike waterbody, network waterbody: together referred to as 'ponds' here on), and the presence of erosion. Elevation (m a.s.l.) and GPS co-ordinates were recorded using a Garmin eTrex 30. Pond size (m^2) was estimated from Google Earth (Google Earth Pro, 2019).

Within each pond, five point measurements of physiochemical conditions were recorded at each sampling depth: pH, water temperature (°C), electrical conductivity (µS), dissolved oxygen (mg/L), and water depth (cm, using a calibrated measuring stick). Water velocity was given a score from 1 (still) to 4 (fast), representing the inflow/outflow.

The vegetation structure and composition were established in nine quadrats of 2 m^2 randomly placed at the water margin. Within each quadrat, plant species, percentage plant cover, and average plant height (using a graduated measuring pole) were recorded.

2.2.3 Dragonfly sampling

Dragonfly (Odonata) sampling was done on sunny windless and cloudless days between 10h00 and 15h00 when dragonfly activity is at its peak. Adult male dragonflies were recorded for 30 min per site over three 100 m transects running perpendicular to the water body (10 min per transect), noting species identifications and their abundance. Close-focus binoculars were used when identification was difficult, although some specimens were collected with a butterfly net and identified using field guides (Samways 2008; Tarboton and Tarboton 2015; Samways and Simaika 2016). Caught individuals were either kept as voucher specimens or released after identification. Voucher specimens included 3 to 4 individuals of a species, which were soaked overnight in acetone to preserve colouration and stored in envelopes which are kept in a reference collection at the Stellenbosch University Entomology Museum.

2.2.4 Aquatic insect sampling

Five 4 m^2 quadrats were chosen to sample at the water margin. This often included the inflows/outflows of the wetland to encompass all microhabitats of the pondscape. The quadrats were swept with an aquatic net (300 mm x 300 mm; 1000 micron mesh) for 3 min each. The net was pushed vigorously into the water to dislodge insects from macrophytes and substrate, ensuring that all the possible microhabitats of the pond margin were reached. The focus was on the pond margin as aquatic insect diversity decreases with the water depth (Samways et al. 1996). The sample was emptied into a large tray for sorting. Target specimens were collected, preserved, and stored in 80% ethanol. Collected individuals were identified at least to genus using the Water Research Commission identification guides (Reavell 2003; Stals 2003; Wilmot 2003) and other relevant field guides (Griffiths et al. 2015).

2.2.5 Frog sampling

Frog calls were recorded between sunset and sunrise, during the peak in their activity. Passive recording was used, whereby pre-programmed recorders were positioned at a central point at the site to record the soundscape. The song meter was positioned on a raised platform so that it was protected from water and above most of the vegetation. The recorder was programmed to record the soundscape for 10 min every hour from 19h00 to 04h00 for 2 days producing a total of 20 recordings per site per season (total of 880 recordings over two seasons). Sample rate of the recordings was at 48 kHz, and the files were saved as audio files. Song meters were moved after 2 days to new sites, allowing for all the sites to be recorded, and with each site having sufficient call data.

Recordings were analysed using Raven Pro 1.6.1 (Center for Conservation Bioacoustics 2019). Spectrograms between 0 kHz and 20 kHz were produced from each recording to aid in the identification of acoustic species. Frog calls range between 0 kHz and 6 kHz, thus any calls above 6 kHz were ignored for this analysis. Calls were identified using a relevant field guide (du Preez and Carruthers 2009). Making use of visual observable patterns within the spectrograms and listening to the individual calls increased familiarity with species allowing for more accurate identifications.

Abundance could not be determined as it is not possible to accurately calculate abundance from call data. Thompson et al. (2020) demonstrates success with using an acoustic activity index (AI) as a proxy for abundance. Thus, the AI from Thompson et al. (2020) was adapted and used to estimate frog species abundance whereby the maximum abundance score is 20, as there were 20 recordings per site per season. The number of recordings in which a single species calls per site reveals its abundance score for data analysis.

2.2.6 Data analyses

PRIMER version 6 (Clarke and Gorley 2006) was used to determine whether the sample sufficiently represents the sampling area. Two effective non-parametric species incidence-based estimators were calculated: Chao2 and Jacknife2 and compared with the number of species observed (Sobs). The Chao2 estimator is successful in insect studies and considers rare species while Jacknife2 reduces the effects of bias.

Species richness was calculated for each taxon (adult dragonflies, beetles, bugs, dragonfly larvae, frogs, plants, and all taxa combined) per sampling site. The Shannon diversity index (subsequently referred to as 'diversity'), which describes species abundance and evenness, was calculated for each taxon (adult dragonflies, beetles, bugs, dragonfly larvae, frogs, plants, and all taxa combined) at each sampling site. This was performed in R using the *vegan* package Oksanen et al. 2017; R Core Team 2020) and then log-transformed.

Spatial testing was performed in R using the *ade4* package (Dray and Dufour 2007; R Core Team 2020) where the Mantel test was used to test for spatial autocorrelation. This was done to check if spatial proximity accounts for any patterns in species richness and diversity of each taxon.

Correlations between the taxa (adult dragonflies, beetles, bugs, dragonfly larvae, frogs, plants, and all taxa combined) were performed in R (R Core Team 2020) using Spearman's correlation coefficient. Correlations were performed on species richness and diversity of each taxon. Correlations between species compositions were performed in PRIMER version 6 (Clarke and Gorley 2006). The

abundance data of each taxon was square-root transformed and converted into resemblance matrices where the Bray-Curtis similarity measure was used to evaluate species composition for each taxon. Correlations were then run between using Spearman's correlation coefficient.

2.2.6.1 Factors influencing species richness, diversity, and abundance

Thirteen environmental variables were tested for multicollinearity to determine which variables should be chosen to describe species richness, diversity, and abundance patterns. These environmental variables were elevation, erosion, soil type (sand or clay), pond size, water velocity, pH, electrical conductivity, dissolved oxygen, water temperature, water depth, % plant cover and average plant height. A regression analysis was run through R using the *car* package (Fox and Weisberg 2019; R Core Team 2020) whereby a backwards selection of the most appropriate environmental variables was performed using a variance inflation factor (VIF) of < 5.

Generalised Linear Mixed Models (GLMM) were performed in R (R Core Team 2020) using the *lme4* (Bates et al. 2015) and *MuMIn* (Barton 2020) packages, to determine the effect of the chosen environmental variables on species richness and diversity for each taxon. Poisson distribution was used to determine the environmental effects on species richness per taxon and Gamma distribution was used to determine the environmental effects on diversity. Linear mixed models (LMM) were used to determine the environmental effects on each endemic species abundance as these data were normally distributed. Model averaging was performed in R (R Core Team 2020) using model selection returned by dredging the models. Model selection was based on Akaike information criterion (AICc) to determine the best fit. For all models, 'plantation' was used as a random spatial factor.

2.2.6.2 Factors influencing species composition

PRIMER version 6 (Clarke and Gorley 2006) was used to perform distance-based linear modelling (DistLM) to explain patterns in species composition using the chosen environmental variables. The abundance data of each taxon was square-root transformed and converted into resemblance matrices where the Bray-Curtis similarity measure was used to evaluate species composition for each taxon. Permutational multivariate analyses of variance (PERMANOVA) with 9999 permutations were used to determine the effects of environmental variables on the species composition within each taxon. The permutational analyses allows for the selection of the environmental variables that explain species composition the best per taxon. Forward selection of the environmental variables was used whereby the environmental variables are added to the model until they no longer showed a significant

effect on species composition. Plantation was used as a fixed factor where Faber's Hill, Good Hope and Mount Shannon were recorded as one plantation due to their close proximity.

2.2.6.3 Identifying a potential surrogate species

Potential surrogate species were determined for three groups: Odonata adults, aquatic insects (Coleoptera, Hemiptera and Odonata larvae), and frogs. The umbrella index (UI) was used to determine the potential of each species within its relative group to act as a surrogate for that group. Calculations were done separately for each group. The umbrella index is based on the sum of three sub-indices: (1) mean percentage of co-occurring species, (2) degree of ubiquity, and (3) sensitivity to human disturbance (Fleishman et al. 2001; Kietzka et al. 2019). Kietzka et al. (2019) was followed for the calculations of the three sub-indices. For Odonata adult species, the Dragonfly Biotic Index (DBI) scores were used to calculate the DSI score (sensitivity to human disturbance). For the aquatic insects, sensitivity scores were scaled from the South African Scoring System (SASS) (Dickens and Graham 2002). There are currently no published sensitivity scores for frogs, thus a scoring system was made for them following a similar scoring system to that of the DBI whereby the sensitivity value is based on three sub-indices: (1) geographical distribution, (2) threat status, and (3) sensitivity to anthropogenic disturbance. Each sub-index ranges from 0 to 3, thus the sum of the three scores produces a score ranging from 0 to 9 where a common, not-threatened, highly tolerant species would score closer to 0 and a range-limited, highly threatened, sensitive species would score closer to 9 (Samways and Simaika 2016). The scoring system for sensitivity to anthropogenic disturbance is as follows: 0 – not sensitive, may thrive in artificial waterbodies, 1 – low sensitivity, common in artificial waterbodies, 2 – medium sensitivity, uncommon in artificial waterbodies, 3 – highly sensitive, only occurs in undisturbed natural habitat. Potential surrogate species were identified as those with a UI score which exceeds the mean UI score for the group plus one standard deviation (Kietzka et al. 2019).

McNemars's *Q*-test (1974), a non-parametric Chi-squared test, was used to determine the proportion of species protected when protecting the identified potential surrogate species. This test was done to compare the proportion of species protected when using a surrogate species from the same group vs. the other groups (Odonata surrogate species for Odonata vs. Odonata surrogate species for frogs and aquatic insects; aquatic insect surrogate species for aquatic insects vs. aquatic insect surrogate species for Odonata and frogs; frog surrogate species for frogs vs. frog surrogate species for Odonata and aquatic insects). Species proportions were sorted into a 2x2 contingency table of the selected surrogate species vs. the beneficiary group (overall assemblage from the same group or overall assemblage from another group). Opposite groups have greater power when their species proportion

are similar versus the comparisons between opposite groups with dissimilar species proportions (Wacholder and Weinberg 1982).

Linear mixed models (LMM) were used to determine the environmental effects on each suite of surrogate species (dragonflies, aquatic invertebrates, and frogs) abundance using the *lme4* (Bates et al. 2015) and *MuMIn* (Barton 2020) packages in R (R Core Team 2020). Model averaging was then performed using model selection returned by dredging the models. Model selection was based on Akaike information criterion (AICc) to determine the best fit. For all models, 'plantation' was used as a random spatial factor.

2.2.6.4 Ponds for conservation

Abundance based rarefaction curves were plotted for each taxon using the *vegan* package in R (Oksanen et al. 2017; R Core Team 2020). Three conservation targets were then used to investigate how many ponds would need to be conserved to adequately conserve the taxa. These conservation targets are defined by their slopes of the abundance based rarefaction curve i.e. slopes of 0.05 (1 new species per 20 sites), 0.1 (1 new species per 10 sites), 0.3 (1 new species per 3.33 sites).

2.3 Results

A total of 28 adult dragonfly species were sampled (531 individuals) with 2 species recognized as national endemics: *Allocnemis leucosticta* and *Pseudagrion caffrum*. *A. leucosticta* was present at 6 sites (out of 22), with a total of 10 individuals, and *P. caffrum* was present at 10 sites (out of 22) with a total of 38 individuals. There was a total of 75 species of aquatic insects (2808 individuals): 16 aquatic bugs (970 individuals), 39 aquatic beetles (762 individuals), 20 dragonfly larvae (1076 individuals). A total of 17 frog species were identified (1509 AI calls) with one endemic frog species (*Leptopelis xenodactylus*) found at 3 different sites (out of 22) with a total of 14 AI calls. Each rarefaction curve reached an asymptote which indicates the sampling conducted was sufficient and reflected the species present, with the exception of beetles (Appendix A and B). This is supported by the species estimates where the number of observed species (Sobs) is near to the estimated number of species (Chao2 and Jacknife2) (Table 2.1).

Table 2.1. Abundance, number of observed species (Sobs) and two species estimators, Chao2 and Jacknife2 per group

Taxon	Abundance	Sobs	Chao2	Jacknife2
Dragonfly adults	531	28	36 (± 11,66)	34.59
Beetles	753	36	40.08 (± 4.55)	43.85
Bugs	970	16	16	16
Dragonfly larvae	1076	18	18.67 (± 1.31)	19.13
Frogs	1509	17	17 (± 14.15)	26.36
Plants	2086	34	47.5 (± 12.46)	48.18
All taxa combined	6925	149	177.04 (± 14.32)	188.06

2.3.1 Correlations between taxa

In terms of species diversity (Table 2.2), dragonfly adults had a significant positive correlation with aquatic beetles and bugs. Within the aquatic insects, beetles had a significant positive correlation with dragonfly larvae. Endemic species had a significant negative correlation with bugs, and overall species diversity. Frogs demonstrated a significant negative relationship with plants. In terms of species richness, bugs had a significant positive correlation with dragonfly adults and larvae and beetles (Table 2.2). Frogs had a significant negative correlation with plants.

In terms of species composition, all taxa showed strong positive relationships with overall species composition with the exception of the endemic species (Table 2.3). Dragonfly adults demonstrated a significant weak positive relationship with beetles and bugs. Within the aquatic insects (beetles, bugs, and dragonfly larvae), each group weakly correlated with one another. Frogs showed a weak positive relationship with beetles and bugs. The species composition of aquatic plants demonstrated weak positive correlations with the aquatic invertebrates and frogs. The endemic species had a weak positive response to bugs and dragonfly larvae (Table 2.3).

Table 2.2. Spearman's correlation coefficient for Shannon's diversity index above the grey midline, Spearman's correlation coefficient for species richness below the grey midline.

	Dragonfly adults	**Beetles**	**Bugs**	**Dragonfly larvae**	**Frogs**	**Plants**	**Endemics**	**All taxa**
Dragonfly adults	1	**0.442***	**0.571****	0.035	0.008	-0.068	-0.319	**0.523***
Beetles	0.406	1	0.41	**0.448***	0.049	0.063	-0.007	**0.587****
Bugs	**0.473***	**0.486***	1	0.249	-0.277	0.215	**-0.593***	**0.77*****
Dragonfly larvae	0.178	0.297	**0.439***	1	0.16	-0.05	-0.354	**0.431***
Frogs	-0.038	0.112	-0.076	-0.049	1	**-0.465***	0.047	0.38
Plants	-0.001	0.075	0.193	0.226	**-0,49***	1	-0.123	**0.436***
Endemics	-0.059	-0.173	**-0.472***	-0.003	-0.2	0.002	1	**-0.62****
All taxa	**0.589****	**0.778*****	**0.82*****	**0.565*****	-0.037	0.34	-0.346	1

Significant correlations are in bold. Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Table 2.3. Spearman's correlation coefficient for species composition.

	Dragonfly adults	**Beetles**	**Bugs**	**Dragonfly larvae**	**Frogs**	**Plants**	**Endemics**	**All taxa**
Dragonfly adults	1	**0.246****	**0.148***	0.158	0.173	0.15	0.084	**0.458*****
Beetles		1	**0.34*****	**0.223****	**0.193****	**0.239****	0.063	**0.533*****
Bugs			1	**0.131***	**0.152***	**0.177***	**0.22***	**0.469*****
Dragonfly larvae				1	0.076	**0.145***	**0.212***	**0.423*****
Frogs					1	**0.189***	0.096	**0.48*****
Plants						1	-0.04	**0.747*****
Endemics							1	0.091
All taxa								1

Significant correlations are in bold. Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

2.3.2 Factors influencing species richness, diversity, and abundance

Dragonfly adult diversity showed a positive significant relationship with water depth (Table 2.4). *Pseudagrion caffrum* (an endemic damselfly) abundance decreased with increasing water depth, and *Allocnemis leucosticta* (also an endemic damselfly) abundance showed a preference for a sandy soil type (Table 2.4). Frog species richness and diversity demonstrated a decrease when there was erosion at the site. Frog diversity decreased with increasing water temperature. *Leptopelis xenodactylus* (an endemic frog) abundance decreased with increasing water depth and dissolved oxygen (Table 2.4). Aquatic plant species richness and diversity increased when the sites were eroded. Species richness and diversity of aquatic plants were higher for sandy soil. Aquatic plant species diversity decreased with increasing dissolved oxygen (Table 2.4). When overall species richness and diversity were assessed, species richness increased with increasing water temperature, species diversity increased with increasing electrical conductivity and decreased with increasing plant height. Overall species richness and diversity increased with increasing water depth (Table 2.4). Aquatic bugs, beetles and dragonfly larvae did not show a significant response to any environmental gradient.

Table 2.4. Environmental variables influencing species richness, diversity, and abundance. The listed species are national endemics.

Taxon	Environmental variable	Species richness	Shannon's index	Abundance
Dragonfly adults	Water depth		(+) 2.615**	
Pseudagrion caffrum	Water depth			(-) 2.247*
Allocnemis leucosticta	Soil			(-) 2.189*
Frogs	Erosion	(-) 2.167*	(-) 4.478***	
	Water temperature		(-) 1.987*	
Leptopelis xenodactylus	Water depth			(-) 2.156*
	Dissolved oxygen			(-) 1.994*
Plants	Erosion	(+) 2.392*	(+) 2.254*	
	Soil	(-) 2.372*	(-) 2.17*	
	Dissolved oxygen		(-) 2.31*	
All taxa combined	Water temperature	(+) 2.113*		
	Water depth	(+) 2.262*	(+) 2.361*	
	EC		(+) 2.008*	
	Plant height		(-) 2.071*	

Value shown is z test statistic. (+): positive correlation; (-): negative correlation. Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

2.3.3 Factors influencing species assemblages

Of the nine environmental variables measured, only five were selected by distance-based linear modelling (DistLM) as significant. These were erosion, water depth, dissolved oxygen, electrical conductivity, and plant height (Table 2.5). For overall adult dragonfly species composition, 10.2% variation was explained by percentage plant cover. The variation in species composition for dragonfly larvae was explained by dissolved oxygen (15.2%) and water depth (1.7%) (Figure 2.2). For overall aquatic plant species composition, 23.6% of the variation was explained by erosion and 9.5% of the variation explain by electrical conductivity (Figure 2.2). Erosion also explained 14.5% of the variation in beetle species composition and 15.7% of overall species composition. Water depth explained 15.1% of bug species composition. Dissolved oxygen explained 16.7% of the variation in frog species composition.

Table 2.5. Environmental variables most descriptive of species composition determined by distance-based linear modelling (DistLM)

Taxon	Environmental variable	F	Variation explained (%)	Cumulative variation explained (%)
Dragonfly adults	% Plant cover	2.281*	10.24	10.24
Beetles	Erosion	3.396**	14.51	14.51
Bugs	Water depth	3.558**	15.1	15.1
Dragonfly larvae	Dissolved oxygen	3.538 **	15.17	15.17
	Water depth	2.762*	10.65	10.65
Frogs	Dissolved oxygen	3.998**	16.66	16.66
Plants	Erosion	5.138**	23.58	23.58
	EC	3.59**	9.5	33.08
All taxa	Erosion	3.732***	15.73	15.73

Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

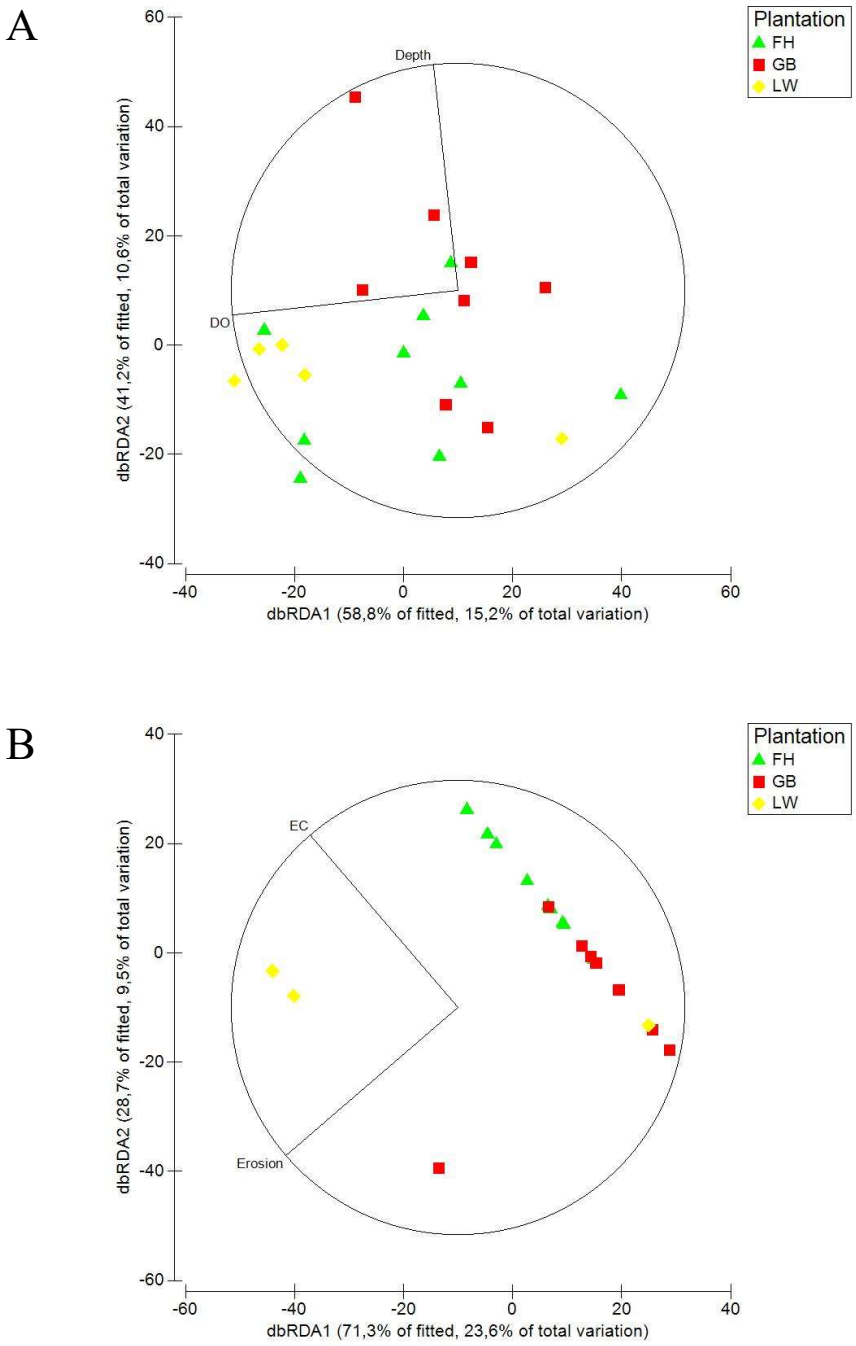

Figure 2.2. Distance-based redundancy analysis (dbRDA) results indicating significant effects of environmental variables on A: Dragonfly larvae and B: Aquatic plants

2.3.4 Identifying a potential surrogate species

For Odonata, five species had umbrella index scores which ranked above the minimum required score to be considered a surrogate species (1.58) (Table 2.6; Figure 2.3). The top ranked odonate adult species was *Pseudagrion caffrum*, which is an endemic species and overlapped with 46% of total Odonata occurrence. Only the top three ranked Odonata species were needed to represent 100% of the Odonata species across the 22 sites (*P. caffrum*, *Zosteraeschna minuscula, P. spernatum*). The suite of surrogates representing adult dragonflies did not significantly respond to any environmental gradients. For the aquatic insects, 10 species had umbrella index scores which ranked above the minimum required score to be considered a surrogate species (1.63) (Table 2.6; Figure 2.3). These included four coleopteran species, two hemipteran species, and four species of Odonata larvae. The top ranked species was a beetle in the genus *Elodes* and overlapped with 55% of total aquatic invertebrate occurrence. Only the top two ranked aquatic invertebrate species were needed to represent 100% of the aquatic invertebrate species across the 22 sites (*Elodes* sp. and *Zosteraeschna* sp.). The suite of surrogates selected to represent the aquatic insects demonstrated a significant increase in abundance with increasing water temperature (Table 2.8).

In the case of the frogs, three species had umbrella index scores which ranked above the minimum required score to be considered a surrogate species (1.61) (Table 2.6; Figure 2.3). The top ranked species of the frogs, *Leptopelis xenodactylus*, is an endemic species and overlapped with 16% of total frog occurrence. All three species were needed to represent 100% of the frog species across the 22 sites. The suite of surrogates selected to represent the frogs demonstrated a decrease in abundance when there was erosion and also decreased with increasing water depth and temperature (Table 2.8). There was no significant difference between the protection levels of same-group vs. opposite-group surrogates for Odonata, aquatic insects, and frogs (Table 2.7). A similar pattern of protection levels was seen where the top three ranked Odonata species represented 100% of the aquatic insects and frogs, the top two ranked aquatic insect species represented 100% of the Odonata and frogs, all three surrogate frog species were needed to represent 100% of the Odonata and aquatic insects.

Table 2.6. Ranked umbrella index (UI) scores of species with UI scores above the minimum required score to be considered an umbrella species (Mean UI + 1 SD).

Species	Mean	Lowest score required	UI score	Cumulative TO %
Odonata adults	1.34 (± 0.23)	1.58		
Pseudagrion caffrum			1.82	46
Zosteraeschna minuscula			1.75	81
Pseudagrion spernatum			1.64	100
Allocnemis leucosticta			1.62	
Chlorolestes fasciatus			1.59	
Aquatic insects	1.45 (± 0.18)	1.63		
Elodes sp.			2.07	55
Zosteraeschna sp.			1.85	100
Ochthebius sp.			1.81	
Lestes sp.			1.78	
Neomacrocoris sp.			1.77	
Hydraena sp.			1.74	
Anax sp.			1.74	
Chlorolestes sp.			1.69	
Hydaticus sp.			1.64	
Frogs	1.37 (± 0.23)	1.61		
Leptopelis xenodactylus			1.95	16
Cacosternum nanum			1.69	72
Strongylopus grayii			1.62	100

Species in bold are endemic.

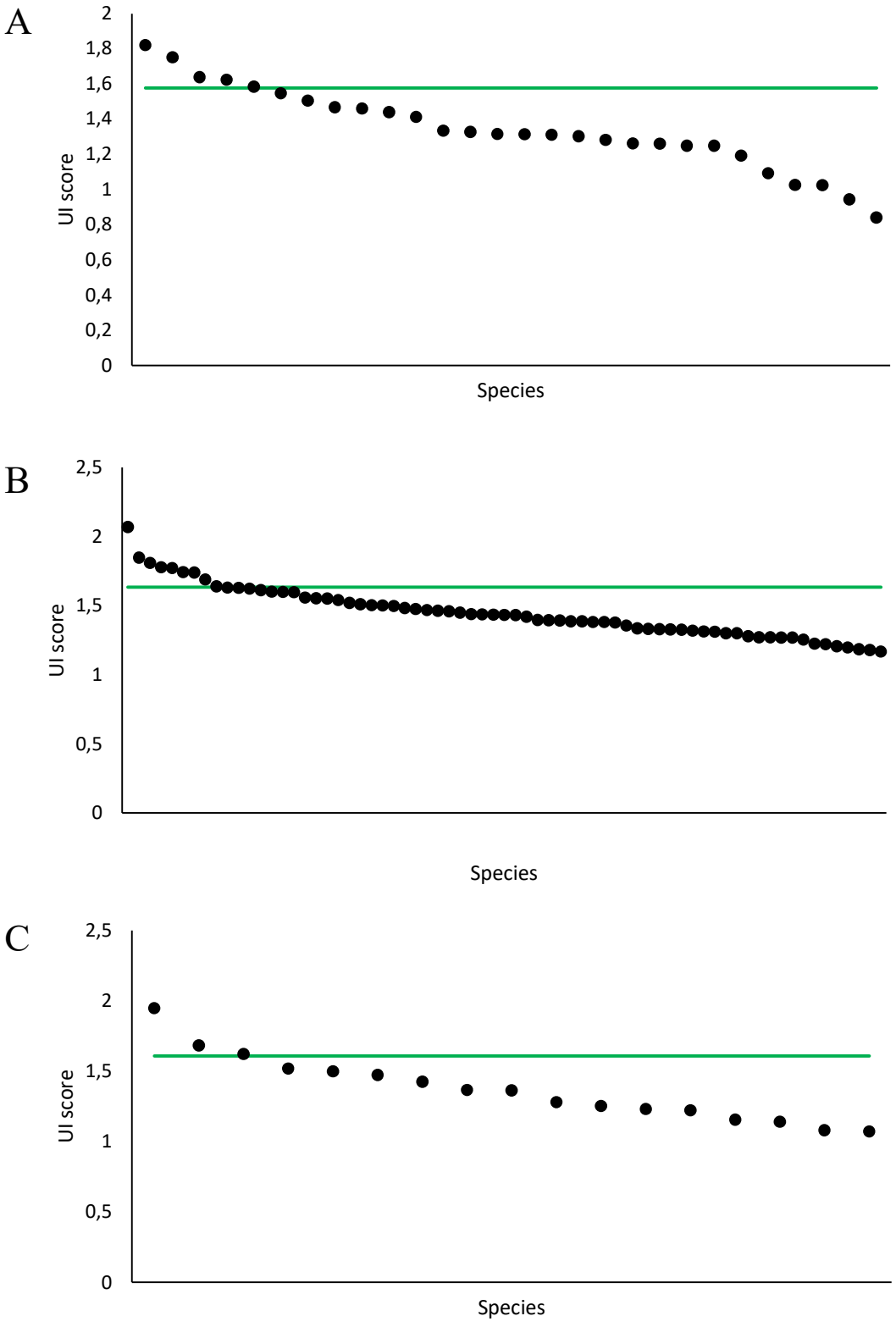

Figure 2.3. Ranked UI scores for A: Dragonfly adults, B: aquatic invertebrates and C: frogs. The horizontal green line represents the minimum score required to be considered an umbrella species (Mean UI + 1 SD)

Table 2.7 Same-group vs. Cross-group surrogate potential

Surrogate	Proportion of species protected		
	Odonata	Aquatic insects	Frogs
Odonata adults			
Pseudagrion caffrum	46	42	44
Zosteraeschna minuscula	81	81	81
Pseudagrion spernatum	100	100	100
Aquatic insects			
Elodes sp.	53	54	55
Zosteraeschna sp.	100	100	100
Frogs			
Leptopelis xenodactylus	10	8	16
Cacosternum nanum	62	61	72
Strongylopus grayii	100	100	100

Table 2.8. Environmental variables influencing abundance of suite of chosen surrogates
Value shown is z test statistic. (+): positive correlation; (-): negative correlation.

Taxon	**Environmental variable**	**Abundance**
Frogs	Erosion	(-) 2.17*
	Water temperature	(-) 2.76**
	Water depth	(-) 2.03**
Aquatic insects	Water temperature	(+) 1.99*

Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

2.3.5 Ponds for conservation

As dragonfly adults are highly mobile, I found a steeper gradient of 0.1 showed that 20 ponds should be conserved in order to adequately conserve dragonfly adults. At the steepest gradient of 0.3, I found that 20 ponds should be conserved in order to adequately conserve beetles. Bugs, dragonfly larvae, frogs, plants, and endemic species all yielded the number of ponds required to conserve them at a slope of 0.05, the flattest slope.

Table 2.9. The number of ponds required to sample for accumulation curves per taxa to flatten to the respective slopes (0.05, 0.1, 0.3) representing 3 different conservation targets (1 new species per 20 ponds, 1 new species per 10 ponds and 1 new species per 3.33 ponds)

Taxon	0.05	0.1	0.3
Dragonfly adults	-	20	10
Beetles	-	-	20
Bugs	7	5	3
Dragonfly larvae	17	12	7
Frogs	15	11	6
Plants	12	11	7
Endemics	5	4	2

2.4 Discussion

2.4.1 Cross-taxon congruence

Cross-taxon congruence in species richness, diversity and composition was weak ($r < 0.57$) and highly variable ($r = 0$-0.57) which yields similar results of several previous studies which aimed to test the cross-taxon congruence amongst riparian and aquatic communities (Heino 2010; Kirkman et al. 2011; Rooney and Bayley 2012; Tonkin et al. 2016; Roque et al. 2017; Valente-Neto et al. 2018; de Morais et al. 2018; Carneiro et al. 2019). Lovell et al. (2007) suggests congruency values greater than 0.75 should be used as the optimal level of congruency when selecting a surrogate, and Heino (2010) suggests that congruence values of 0.70 or greater should be considered for conservation planning. De Morais et al. (2018) emphasize the errors associated with defining an optimal cross-taxon congruence value, whereby some studies consider strong congruency to be represented by significant correlation, while other studies focus on the strength of the correlation, but de Morais et al. (2018) also found statistical significance alone to be insufficient to determine concordance. Assemblages tend to respond differently to environmental gradients and biotic interactions, especially at finer spatial scales. This means that levels of congruence would seldom be high enough to agree with a defined optimal cross-taxon congruence value (Heino et al. 2007; Qian and Ricklefs 2008; Padial et al. 2012; Tonkin et al. 2016; de Morais et al. 2018). It is suggested that if a single assemblage predicts the patterns of several other assemblages, then it should be considered as a potential surrogate group regardless of low congruency values (Padial et al. 2012; Carneiro et al. 2019).

Previous studies show that adult dragonflies are strongly congruent with macroinvertebrates (Smith et al. 2007 (in rivers); Magoba and Samways 2010 (in rivers); Briggs et al. 2019a (in ponds)) and are suggested to be an important surrogate for freshwater systems at large spatial scales (Samways and Simaika 2016). Measures of adult dragonfly species diversity and species composition demonstrated a significant relationship with aquatic beetles and bugs, which was expected due to their similarly high dispersal abilities. The correlations are significant, but the r values are too low to reach the optimal cross-taxon congruence values outlined by Lovell et al. (2007) and Heino (2010). Dragonfly adult diversity increased as water depth increased, a similar trend as seen in aquatic bug species composition (also seen in dragonfly larvae species composition and overall species richness and diversity). Most aquatic beetles and bugs tend to occupy the shallow margins of the pond as there is a reduction in temperature and dissolved oxygen combined with a decrease in aquatic invertebrate richness and abundance when water depth exceeds 1.2 m (Samways et al. 1996). However due to the drought experienced by KwaZulu-Natal in 2019 (Agri SA 2019), the water depths of the ponds studied ranged between 7-62 cm, thus the increase in species composition and diversity associated with increased water depth is likely a response to escape drying out. Female adult dragonflies are responding to water depth due to oviposition preference, confirmed by a similar response seen in dragonfly larval species composition. Assemblages which have similar responses to the same environmental variables have been recorded as one of the main drivers of cross-taxon congruence (Gioria et al. 2010; Padial et al. 2012; Carneiro et al. 2019), but my results only show a similar response to water depth between dragonfly adult composition and aquatic bug diversity, which was consequently seen in overall species richness and diversity.

Previous studies have demonstrated strong congruence between dragonfly adults and larvae (Smith et al. 2007; Mendes et al. 2017) at moderate to large spatial scales (Kietzka et al. 2019), but my results revealed weak congruence between adults and larvae, similar to Valente-Neto et al. (2016, 2018). The dissimilar species composition patterns observed between adults and larvae is likely due to differing environmental requirements as dragonfly larvae are limited to the aquatic environment. This is illustrated by the response of dragonfly larvae to dissolved oxygen, required for respiration, unlike air breathing aquatic beetles and bugs and terrestrial adults (Griffiths et al. 2015; Bird et al. 2019). Dragonfly adult species composition more readily responded to percentage of plant cover which is not surprising as dragonflies require marginal or submerged vegetation to perch, as refugia for larvae, for emergence sites and for food (Samways and Simaika 2016; Deacon et al. 2018). It is also widely noted that dragonfly adults demonstrate a rapid response to change in marginal vegetation (d'Amico et al. 2004; Golfieri et al. 2016; Samways and Simaika 2016; Valente-Neto et al. 2016; Briggs et al. 2019a).

Within the aquatic insects, the composition of aquatic beetles, bugs, and dragonfly larvae were correlated; however, cross-taxon correlations are significantly moderate (0.13 – 0.49), but do not reach the optimal cross-taxon congruence values outlined by Lovell et al. (2007) and Heino (2010). There was no shared dependence on an environmental variable aside from water depth, already attributed to a response to drought, thus concordance between the aquatic insects is too weak for consideration of a surrogate. This disagrees with previous studies where concordance is seen within macroinvertebrates due to similar environmental pressures. For example, Bilton (2006), demonstrated high congruence between aquatic beetles and other aquatic insects and even recommends beetles as a surrogate taxon for rapid assessment of pond diversity.

Beetle species composition demonstrated a significant positive response to erosion. Eroded sites tended to have a high water velocity. Aquatic beetle environmental preferences are highly variable, and they can be found in lentic and lotic waters (Bird et al. 2019), thus the underlying influence may be biotic interactions. Prey diversity (Ephemeroptera) was much higher in ponds with a higher water velocity which is consistent with mayfly preference for fast-flowing streams (majority of species) (Vilenica et al. 2017), which may drive a higher aquatic beetle predator population. A significant correlation was seen between aquatic plant and beetle species composition, with aquatic plant species composition also showing a positive response to erosion. The r value (0.24) was too low to demonstrate congruency, but aquatic plants had the highest correlation with beetles which is attributed to vegetation requirements for hiding and predation by aquatic beetles (Gioria et al. 2010) and the shared response to erosion.

Plant species composition revealed weak, but significant relationships with aquatic beetles, bugs, dragonfly larvae and frogs and were strongly congruent with overall species composition demonstrating their potential use as a surrogate corresponding to previous studies (Gioria et al. 2010), but this is tentative due to plants being partially correlated with themselves. The concordance in species composition is due to increased microhabitat heterogeneity from increased plant species composition and biotic relationships. Aquatic beetles and bugs, dragonfly larvae and frogs all depend on plants, directly or indirectly, for food, shelter and/or refugia, as well as microhabitats for ambush predation and perches (Gioria et al. 2010; Briggs et al. 2019b). No significant relationship was seen between plants and adult dragonflies, consistent with previous studies (Bried et al. 2007), which illustrates dragonfly response to vegetation structure above vegetation species composition due to perching and shade preferences (Samways and Simaika 2016).

Plant species richness, diversity and composition all demonstrated a significant positive correlation with erosion attributed to increased invasion by alien plants in eroded sites. Erosion created a

disturbance at those sites, allowing for the invasion of alien plant species which causes a false inflation in species richness and complexity (Catford et al. 2012) as alien vegetation generally causes a reduction in community complexity and heterogeneity. Plant preference for erosion was shared with aquatic beetle species composition and overall species composition which may have been driven by plant structure. Overall species diversity decreased with increasing plant height which is expected as shading of the water resulting from tall marginal vegetation causes dragonfly and macroinvertebrate impoverishment (Samways and Sharratt 2010; Samways et al. 2011)

In terms of species richness and diversity, frogs showed no significant relationships, thus they cannot be used as an indicator taxon as hypothesized. Frog species composition showed weak, but significant relationships with aquatic beetle and bug species composition. It is likely that aquatic insects have higher dispersal capabilities than frogs and can more easily fly to a new pond when conditions are not optimal. Frog populations are highly dynamic, fluctuating drastically with annual variations in climate (Semlitsch et al. 1996) and tend to exist as metapopulations across the pondscape (Kirkman et al. 2012). This creates a similar dispersal pattern to that of mobile aquatic beetles and bugs that fly between ponds on the basis of habitat preference but may be more seasonal in the case of frogs.

I hypothesised that frogs would show cross-taxon congruence with riparian and aquatic communities and would be successfully used as a surrogate group, but this was not the case. There are no cross-taxon correlations strong enough to identify a potential surrogate. I recommend a multi-taxon approach, increasing spatial scale at which congruency is tested. Cross-taxon congruence is often attributed to shared reliance on environmental gradients, but my results showed very little shared reliance on the environmental gradient between taxa.

2.4.2 Umbrella species concept

Although wetlands are heterogenous and highly variable environments, I was able to identify a suite of surrogate species to represent their taxa. There was no cross-taxon congruence, thus only same-group surrogate species should be considered when protecting wetland species assemblages.

The umbrella index maximises median rarity which means that it can identify fewer locations for protection without sacrificing protection of species, allowing for efficient site selection (Fleishman et al. 2001). It is a strength of the umbrella index to focus less on rare and endemic species, as endemic species are not always correlated with high species richness. This is illustrated in my results where species richness was comparatively lower where the endemic frog *Leptopelis xenodactylus* was recorded. It still included rare species, ensuring their protection. These species scored lower in the median rarity sub-index, but their high sensitivity to environmental change and endemism included

them as surrogates. This was the case for frog surrogate species, *L. xenodactylus,* adult dragonfly species, *A. leucosticta* and dragonfly larval species, *Chlorolestes fasciatus*. Other surrogate species showed less sensitivity to disturbance but co-occurred with many other species (had median rarity), thus had high scores. This was evident in the adult dragonflies where *P. spernatum* (DBI score = 3) ranked above *A. leucosticta* and *C. fasciatus*, which have higher DBI scores (5 and 4 respectively). The majority of the species selected by the umbrella index were selected for being sensitive to human disturbance, occurring with many other species and being between rare and median rarity. This allows for the selection of a suite of surrogates that range from rare, highly sensitive, co-occurring species to less sensitive, co-occurring species.

It can be argued that any species occurring across a significant proportion of the region may function as an umbrella species, but a randomly selected group of species would require a larger proportion of the landscape to be designated for conservation to achieve a given level of species protection (Fleishman et al. 2001), meaning the umbrella index maximises species protection per area (Betrus et al. 2005). It is also important that a suite of species be chosen as surrogates for conservation planning rather than one species due to life histories and microhabitat preferences. This study focused on wetlands, which could encompass a variety of pond descriptors, where some ponds were still and temporary and others were permanent, some having significant inflows/outflows. Certain pond types may have certain microhabitat characteristics for the selected surrogates that others do not. For example, the top ranked dragonfly surrogate species, *P. caffrum* prefers feeder streams with riffles (Samways 2008), thus the absence of the species at a still wetland should not necessarily represent a poor quality wetland and abandon the site for conservation. It is important to note heterogeneity within the water ecotone itself as it is often difficult to classify waterbodies as strictly lentic or lotic (Samways and Stewart 1997) where the wetland may support both lentic and lotic microhabitats.

Most of the aquatic insect surrogate species were chosen due to their moderate sensitivity to disturbance (majority had a SASS score of 5), while the top ranked aquatic invertebrate, *Elodes* sp. had the highest SASS score (8). *Elodes* sp. is a beetle only aquatic at its larval stage, preferring still waters, thus representative of the opposite microhabitat preference to that of *P. caffrum*. The use of the entire suite of surrogates would more accurately predict which ponds are to be protected as aquatic beetles, bugs and dragonfly larvae all have a variation in species-specific environmental preferences. The suite of surrogate species selected to represent aquatic invertebrates showed a significant positive response to water temperature. This is response is expected as aquatic beetles and bugs tend to prefer warmer waters and increased temperatures promote decreased larval development time (Bird et al. 2019).

The top ranked frog surrogate species was the Long-Toed Tree frog, *Leptopelis xenodactylus*. The species is highly endemic occurring mainly in the southern KwaZulu-Natal highlands; thus, its use as surrogate species is limited and can only be considered in this region. While site selection was based on known locations of the species, its call was only heard at three of the 22 sites sampled. Although the umbrella index maximises median rarity, *L. xenodactylus* was rare, and its high sensitivity to disturbance, endemism, and threat status is what highlighted it as the top surrogate species for frogs. The suite of species selected to represent frogs illustrated a negative response to erosion, increasing water temperature and increasing water depth. The negative association with erosion and increasing water temperature was seen amongst frogs as a whole, but the negative response to increasing water depth was only demonstrated in *Leptopelis xenodactylus*, also showing a negative response to increasing dissolved oxygen. This highlights frog response to environmental change in both the terrestrial and aquatic systems. The suites of surrogates did not share any environmental responses, with frogs and aquatic insects even showing opposite responses to water temperature. This further confirms that the suite of surrogates chosen cannot be used for cross-taxon groups. Same-group surrogates should only be considered when protecting species assemblages which implicates a multi-taxon approach to selecting surrogates**.**

2.4.3 How many ponds in a conservation pondscape?

A group of ponds forms a network of water bodies across a landscape with high habitat heterogeneity and mosaics of important microhabitats, increasing area of occupancy for many species. Pondscapes support aquatic, semi-aquatic and terrestrial fauna and flora and provide habitat stepping-stones for species to move across the landscape to colonise new ponds (Briggs et al. 2019b; Hill et al. 2018). Ponds do not exist in isolation and are affected by regional and landscape influences, which highlights the requirement for regional management of ponds. My results show that the taxa studied likely exist in metapopulations across the pondscape due to certain ponds only having certain species-specific microhabitat characteristics. Investigation at a larger spatial scale, for example the pondscape level, will be important in understanding how to adequately conserve species assemblages across a region. My results indicate that the conservation of 20 ponds in a pondscape would adequately conserve an assemblage of dragonfly adults, aquatic insects, frogs, and plants. My results showed that certain groups required less ponds to be conserved than others, for example, dragonfly larvae only needed 17 ponds and frogs only needed 15 ponds for adequate conservation, likely due to dispersal capabilities of each group. Highly mobile groups such as adult dragonflies and beetles can easily fly between ponds searching for optimal habitat and would require more ponds in a pondscape to satisfy their habitat requirements. Less mobile groups such as dragonfly larvae are limited to their pond and will likely require less ponds in the pondscape, similarly frogs have lower dispersal capabilities than

that of mobile adult dragonflies and beetles and would span fewer ponds. From my results, I recommend a multi-taxon approach for site selection in conservation management and that at least 20 ponds be conserved as part of a larger pondscape to ensure the conservation of aquatic and riparian communities.

2.5 Conclusions

Cross-taxon congruence was not strong enough to identify a single surrogate group to confer protection to the other focal groups. There were limited patterns of similar response to the same environmental gradients which further implicates weak congruency. The umbrella index can be successfully applied to find a suite of surrogates to represent its own taxon and was fairly flexible with the inclusion of both endemic and rare species and co-occurring, less sensitive species within the suite of surrogates. I recommend using a multi-taxon approach when selecting surrogates for conservation management of a pondscape. I also found that a pondscape should consist of at least twenty ponds to confer adequate protection to the wetland associated species studied here.

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Chapter 3: A viable conservation pondscape includes the ecotones with dryland

Abstract

Wetlands are transitional zones between standing water and terrestrial environments, where the local wetland and dryland species and communities overlap. However, little is known about the assemblage rules of the organisms that inhabit this ecotone. I separated the wetland/dryland ecotone of 22 ponds into five zones according to plant composition and structure, and water depth. I then tested the extent to which insect species assemblages change across these five zones, and the environmental factors that drive these changes. Plants showed a clear pattern across the ecotone with each zone having a characteristic suite of species, but different assemblages across the various ponds. Dragonflies were distributed across the entire ecotone, although peaked in the marsh zones at the ecotone centre, and where marginal vegetation enabled perching and hiding behaviour. Butterflies peaked in the grassland zone, although there were also some marsh-associated species. Grasshoppers were strongly associated with the grassland zone and showed a negative response to increasing water body size. Species associated with the open water, deep marsh, and shallow marsh zones (here called aquatic species), consisted of plants and dragonflies. Aquatic species composition showed a response to erosion, water temperature, plant height, and soil type. Terrestrial species (plants, grasshoppers, butterflies) composition showed a response to erosion, electrical conductivity, and pH i.e., the terrestrial species still responded to water conditions even though they were associated with the dry grasslands. Pond ecotones have high species turnover and high conservation value for both aquatic and terrestrial species, indicating that ponds have a much larger footprint than just the extent of the wet areas. Terrestrial buffer zones are required for efficient protection of wetlands and the biota in general. In sum, conservation pondscapes must include the transition zones which contribute greatly to local heterogeneity both around any one pond and among a set of functionally connected ponds.

Keywords: ponds, transitional zone, beta diversity, species replacement, turnover, plants, insects

3.1. Introduction

Wetlands are transitional zones between open water and terrestrial ecosystems. They form a gradient, or ecotone, between the two habitats. Ecotones are defined as transition zones occurring where the environmental limits of species and communities overlap and can be considered the boundary created between two adjacent and distinct habitats (Holland 1988; Brownstein et al. 2015; Wight et al. 2017). Wetland ecotones are driven by many factors, which need to be understood for informing conservation and management practices (Brownstein et al. 2015). While ecotones can be viewed as overlapping communities, they can also be species-rich and considered as entities in their own right (Dangerfield et al. 2003). Human land-use practices cause an increase in the proportion of edges and

ecotones in landscapes, resulting in a variety of taxon-dependent responses to ecotones (Peyras et al. 2013), an important focal point in habitat management.

Recommendations to date suggest that to protect a wetland there needs to be a terrestrial buffer zone of 30-60 m around it to ensure its quality (Castelle et al. 1994; Semlitsch and Bodie 2003; Roe and Georges 2007). However, the habitat zone for wetland amphibians and reptiles extend 289 m from the water margin plus a further 50 m of a terrestrial buffer zone to account for edge effects, meaning that a 339 m buffer is required to adequately protect wetland species (Semlitsch and Bodie 2003).

Wetland ecotones are also an important habitat for semiaquatic species that rely on both the aquatic and terrestrial habitats for life-history functions (Dangerfield et al. 2003). However, caution is required not to consider just wetland patches but rather focus of the wider landscape as a whole. This is because ecological processes generally depend on both patch and matrix quality, as well as quality linkages between patches, with management practices required to consider entire pondscapes rather than individual ponds or wetlands (Roe and Georges 2007; Chapter 2).

While there has been a focus on terrestrial-wetland or aquatic-wetland boundaries, it is perhaps better to focus on the entire transition from a wet to dry habitat, where ecotone width is determined by adjacent yet distinct plant communities (Kolasa and Weber 1995; Wight et al. 2017). Wetland ecotones can be sharp and narrow or wide and diffused. This translates into abrupt changes in plant diversity and density vs. a wider area with a gradual decrease in macrophyte densities (Kolasa and Weber 1995). The spatial arrangement of vegetation type can strongly influence ecosystem processes and the character of the vegetation zones across the environmental gradient, with water depth often the dominant factor influencing species spatial distribution (Seabloom and van der Valk 2003).

Many animals use multiple microhabitats to fulfil all life functions, and how these habitats are interspersed has a strong effect on population dynamics and long-term viability (Seabloom and van der Valk 2003). However, species responses to ecotones can be difficult to elucidate, and not every taxonomic group responds similarly (Wight et al. 2017). Invertebrate composition changes unevenly and unpredictably across habitat edges, and the degree of change is taxon dependent, with non-pollinating insects not necessarily locally congruent with plants (Dangerfield et al. 2003).

Species richness in ecotones, could on the one hand be low from environmental fluctuation causing stress, while on the other, it will be high as species benefit from access to two adjacent communities (van der Maarel 1976; Liu and Cui 2009; Brownstein et al. 2013; 2015). However, the most commonly reported case is that species richness in wet-/dryland ecotones are intermediate between the two communities, attributable to species being adapted to either wet or dry environments, with

few species spanning the whole moisture gradient (Brownstein et al. 2015). Species richness in wet-/dryland ecotones is enhanced through many species being semiaquatic, in addition to specialist ecotonal species (Dunning et al. 1992; Lloyd et al. 2000), plus risk of tension between the adjacent communities allowing for invasion by exotic species (Lloyd et al. 2000).

Dragonflies are well known indicators of biodiversity within freshwater systems, particularly when used as an index of water quality (Dragonfly Biotic Index) based principally on the presence of adult species to indicate ecological integrity (Samways 2008; Samways and Simaika 2016). Dragonflies are taxonomically well understood in the region, and suitable for ecological assessments. Adult dragonflies show a rapid response to changes in marginal vegetation structure and require a mixture of direct sunlight and shade within proximity of the water for perching and thermoregulation (Samways and Simaika 2016; Valente-Neto et al. 2016; Briggs et al. 2019; Cunningham-Minnick et al. 2019). Terrestrial vegetation structure is also a requirement to support prey populations for adults. Cunningham-Minnick et al. (2019) found dragonfly abundances to be high in ecotone habitats (transitional zone) demonstrating greater sensitivity to riparian and aquatic vegetation than stream or open-water dragonfly communities. They also found the transitional zone to be preferred by breeding individuals and suggest using the ecotone for conservation monitoring of wetland ecosystems.

Butterflies are indicators of high habitat quality in the area, especially presence of high quality nectar resources (Pryke and Samways 2001, 2003; Bazelet and Samways 2012). Zones rich in flowering plants increase butterfly species and corridors are important for survival and dispersal for many butterflies. Wetlands have some habitat specialist butterflies, with floral diversity being important for them (Kyerematen et al. 2014; 2018), as is grass height (Pryke and Samways 2003). Although I expect butterfly species richness and diversity to be highest in grassland zone, water sources, or perhaps a mud puddle, will support extra species in the wet meadow zone. Shady resting areas and sunny patches for basking are also important requirements for butterflies (Dennis and Shreeve 1988).

Grasshoppers are sensitive indicators of vegetation structure and composition (Chambers and Samways 1998; Gebeyehu and Samways 2003; McGeoch 2007; Bazelet and Samways 2011), especially for the dry end of the ecotone, and in this geographical area, are highly congruent with butterflies (Bazelet and Samways 2012).

To define variation in biodiversity among zones, there must be a measure that portrays changes in species composition between local assemblages, i.e. a measure of beta diversity (Whittaker 1960; Anderson et al. 2011; Johansson et al. 2019) which reflects differences in assemblage composition between zones. Beta diversity determines the relationship between local and regional diversity and degree of differentiation among biological assemblages or communities (Baselga 2010) based on

compositional dissimilarity due to differences in species richness (i.e. a nested assemblage) or dissimilarity due to spatial turnover derived from replacement of species (Baselga et al. 2007; Johansson et al. 2019).

Ecotones are context-dependent with various environmental factors determining diversity patterns. Here, I divide a dry-/wetland gradient into five zones according to water depth and dominant vegetation, and focus on dragonflies (being amphibiotic, with aquatic larvae and aerial adults), butterflies and grasshoppers (both having habitats with a range moisture preferences). I measure the change in species assemblages over the aquatic-terrestrial ecotone and determine the drivers of this change through determination of peaks in both species richness and beta-diversity based on the following objectives: 1) Assessment of changes in species richness and assemblage composition per zone (open water, deep marsh, shallow marsh, wet meadow, and dry grassland), as well as beta diversity (partitioned into nestedness and turnover). 2) Assessment of each zone across the pondscape to ascertain whether species assemblage patterns across the ecotone are consistent across the entire pondscape, or whether each pond has its own set of species representing each zone. 3) To identify wet-associated and dry-associated species and assess their responses to environmental variables to determine the drivers of species assemblage patterns across the ecotone. I hypothesise that 1) there will be higher species turnover in the intermediate zones (deep marsh, shallow marsh, and wet meadow) due to overlapping species preferences driven largely by vegetation composition and structure. Furthermore, I hypothesise that 2) each pond will have its own set of species representing vegetation zones due to microhabitat preferences and that 3) wet-associated and dry-associated species will have different environmental drivers, being at opposite extremes of the ecotone.

3.2 Materials and Methods

3.2.1 Study area and design

Study sites were selected from five timber plantation mosaics in the KwaZulu-Natal Midlands. These estates are Faber's Hill, Mount Shannon, Good Hope, Linwood, and Mount Gilboa (Figure 3.1). All study sites were in conservation sectors of the estates and were more than 300 m apart. I selected 22 sites of various wetland and pond types, based on hydrophilic plant species and water body characteristics, and were either on open grassland plains or in shallow valley bottoms. Fieldwork was conducted over two seasons: early summer (between November and December 2019) and late summer (March 2020).

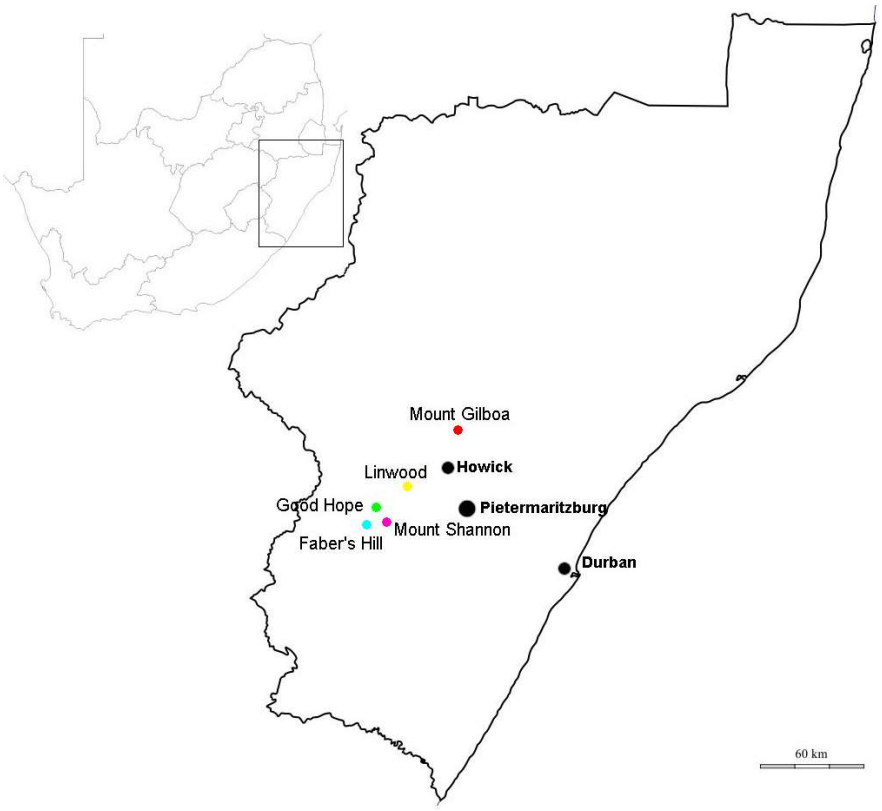

Figure 3.1. Locations of plantations, Faber's Hill (blue), Good Hope (green), Mount Shannon (pink), Linwood (yellow) and Mount Gilboa (red). Nearby towns are in bold

At each site, 3 transects of 100 m were run from open water to grassland. The 100 m transects were divided into five vegetation zones: open water, deep water marsh, shallow water marsh, wet meadow, and dry grassland, together representing the aquatic-terrestrial ecotone. Vegetation zones were characterized by dominant plants and water depth and were variable in size across the 22 ponds. The open water zone was dominated by submerged or emergent aquatic plants, and >25 cm water depth. Deep marsh was tall reeds and bulrushes with a water depth 25-15 cm. Shallow marsh was emergent grasses and sedges, and <15 cm water depth. Wet meadow was hydromorphic soils dominated by shrubs and grasses, and dry grassland was a terrestrial zone dominated by grasses.

3.2.2 Environmental variables

Categorical variables were recorded at the site level to distinguish differences between the types of ponds sampled: soil type and waterbody shape (pond waterbody, stream-like waterbody, network waterbody: together referred to as 'ponds' here on), and presence of erosion. Elevation (m a.s.l.) and

GPS co-ordinates were recorded using a Garmin eTrex 30. Pond size (m^2) was estimated from Google Earth (Google Earth Pro, 2019).

Within each pond, five point measurements of physiochemical conditions were undertaken at each sampling depth: pH, water temperature (°C), electrical conductivity (µS), dissolved oxygen (mg/L), and water depth (cm, using a calibrated measuring stick). Water velocity was given a score from 1 (still) to 4 (fast), representing the inflow/outflow.

Two quadrats of 2 m^2 were randomly placed along the transect within each vegetation zone. Plant species present within each quadrat were identified, and percentage cover by each species per quadrat per zone was recorded. Average vegetation height per zone was also recorded, using a graduated measuring pole.

3.2.3 Invertebrate sampling

Three taxonomic invertebrate groups were studied: dragonflies (Odonata), butterflies (Lepidoptera), and grasshoppers (Orthoptera). Sampling was only of adults to ensure correct identification and was done on sunny windless and cloudless days between 10h00 and 15h00, the activity peak. Adult male dragonflies and butterflies were recorded for 10 min per transect (30 min per site) along the 100 m transects running from open water to grassland. Species identifications and abundances were recorded per vegetation zone. Close-focus binoculars were used when identification was difficult, although some specimens were collected with a butterfly net and identified using field guides (Woodhall 2005; Samways 2008; Tarboton and Tarboton 2015; Samways and Simaika 2016). Caught individuals were either kept, as voucher specimens, or released after identification. Voucher specimens included 3 to 4 individuals of a species, which were frozen overnight, and adult dragonflies were soaked in acetone to preserve colouration and stored in envelopes. Butterflies were pinned and spread. Specimens are kept in a reference collection the Stellenbosch University Entomology Museum.

Grasshoppers were sampled in late summer as I focused on adults, and grasshoppers only mature into adults in late summer. Adult grasshoppers were collected by sweep netting along each 100 m transect running from open water to grassland for 8 min per transect. Grasshoppers collected during sweep netting were placed in a small plastic bag and frozen overnight. Collected specimens were soaked in acetone to preserve colouration, sorted into morphospecies, and their abundances recorded. Voucher specimens of 2-4 individuals of each morphospecies were retained in Stellenbosch University Entomology Museum.

3.2.4 Data analyses

PRIMER version 6 (Clarke and Gorley 2006) was used to determine the effectiveness of sampling where two effective non-parametric incidence-based species estimators were calculated. These are Chao2 and Jacknife2, which were compared with the number of species observed (Sobs). These two estimators were chosen because the Chao2 estimator is successful in insect studies and considers rare species while Jacknife2 reduces the effects of bias.

Test for spatial independence was performed in R using the *ade4* package (Dray and Dufour 2007; R Core Team 2020) using the Mantel test to for spatial autocorrelation. The Mantel test indicated no significant spatial autocorrelation within the data, but 'plantation' was used as a random spatial variable in all linear models to account for any influence from spatial proximity of the wetlands.

GLMMs were performed in R using the *lme4* package (Bates et al. 2015; R Core Team 2020) to determine the effect of ecotone zone on species richness. The models were based on Poisson distribution. Ecotone zone (open water, deep marsh, shallow marsh, wet meadow, and grassland) was included as a fixed variable and plantation a random variable. Once the Chi-squared (χ^2) values were estimated and the effect of zone on species richness were significant, Tukey post hoc tests were performed using the *multcomp* package in R (Hothorn et al. 2008; R Core Team 2020) to determine pairwise differences between the zones.

A permutational multivariate analysis of variance (PERMANOVA) was performed in PRIMER version 6 (Clarke and Gorley 2006) to test for significant differences in species composition between the five zones per taxon. The PERMANOVA was performed using resemblance matrices from the Bray-Curtis similarity measure, using square root transformed abundance data. The test was permutated 9999 times. 'Zone' was used as a fixed factor and 'plantation' was used as a random variable. Where the zones significantly influenced species composition, pairwise comparisons were then performed using PERMANOVA to determine which zones showed significant differences in species composition. To visualise plant, dragonfly, and butterfly species composition among the ecotone zones, Canonical Analysis of Principal coordinates (CAP) were performed in PRIMER version 6 (Clarke and Gorley 2006). Grasshoppers were excluded from multivariate analyses as adults were only recorded in the grassland zone. Although nymphs were found in the wet meadow zone, the study focused on adults, these unidentifiable nymphs were excluded.

Alpha diversity along the ecotone zones was calculated using the *BAT* package in R (Cardosa et al. 2014; R Core Team 2020) to ascertain an average measure of species diversity in each zone for each taxon in both seasons. Overall beta diversity between the ecotone zones was calculated using the *BAT*

package in R (Cardosa et al. 2014; R Core Team 2020), using Bray-Curtis measure of dissimilarity. This was calculated to determine whether the differences in alpha diversity across the ecotone zones is due to species turnover or species richness. Beta diversity was calculated by partitioning beta diversity into its constituent parts of species turnover and change in species richness between zones.

Each zone was investigated individually, and zones compared across the 22 sites to ascertain whether differences in alpha diversity between sites was due to species turnover or a change in species richness. Using the *betapart* package in R (Baselga et al. 2020; R Core Team 2020) beta diversity was performed multiple times for comparisons between sites based on abundance, using the Bray-Curtis dissimilarity measure. This was resampled 100 times, randomly picking 6 sites for each sample. Using the *multcomp* package in R (Hothorn et al. 2008; R Core Team 2020), analysis of variance (ANOVA) was performed on the resampled data to compare differences in beta diversity between the different zones, followed by a Tukey's post hoc test when there were significant differences ($p < 0.05$) between zones.

The *indval* function was run through R using the *indicspecies* package (Cáceres and Legendre 2009; R Core Team 2020), to determine which species were associated with the dry and wet zones per season. Species associated with open water, deep marsh and/or shallow marsh zones were grouped together and here called 'aquatic' species. Species associated with the grassland zone were here called 'terrestrial' species.

Thirteen environmental variables were tested for multicollinearity to determine which variables should be chosen to describe species richness and composition patterns. These environmental variables were elevation, erosion, soil type (sand or clay), pond size, water velocity, pH, electrical conductivity, dissolved oxygen, water temperature, water depth, % plant cover and average plant height. A regression analysis was run through R using the *car* package (Fox and Weisberg 2019; R Core Team 2020) whereby a backwards selection of the most appropriate environmental variables was performed using a variance inflation factor (VIF) of < 5.

Generalised Linear Mixed Models (GLMM) were performed in R (R Core Team 2020) using the *lme4* (Bates et al. 2015) and *MuMIn* (Barton 2020) packages, to determine the effect of the chosen environmental variables on species richness of each taxon per season. Poisson distribution was used to determine the environmental effects on species richness. Model averaging was performed in R (R Core Team 2020) using model selection returned by dredging the models. Model selection was based on Akaike information criterion (AICc) to determine the best fit. For all models, 'plantation' was used as a random spatial factor.

PRIMER version 6 (Clarke and Gorley 2006) was used to perform distance based linear modelling (DistLM) to explain patterns in species composition using the selected environmental variables. Abundance data of each taxon was square-root transformed and converted into resemblance matrices where the Bray-Curtis similarity measure was used to evaluate species composition for each taxon. Permutational multivariate analyses of variance (PERMANOVA) with 9999 permutations were used to determine the effects of environmental variables on the species composition within each taxon. The permutational analyses allows for the selection of the environmental variables that explain species composition the best per taxon. Forward selection of the environmental variables with environmental variables added to the model until they no longer showed a significant effect on species composition. 'Plantation' was used as a fixed factor where Faber's Hill, Good Hope and Mount Shannon were recorded as one plantation due to their close proximity.

3.3 Results

In early summer, a total of 26 dragonfly species and 17 butterfly species were sampled. In late summer, a total of 25 dragonfly species, 29 butterfly species, and 46 grasshopper morphospecies were sampled. There were 114 plant species. Dragonfly and grasshopper species accumulation curves approached an asymptote (Appendix C), confirmed by the species estimators Chao2 and Jacknife2 (Table 3.1). There was one less dragonfly species late compared to early summer. Butterflies and plants did not reach an asymptote, further confirmed by the high species estimates (Table 3.1).

Table 3.1. Abundance, number of observed species (Sobs) and two species estimators, Chao2 and Jacknife2 per group over two seasons

Taxon	Sobs	Chao2 (±SE)	Jacknife2
Early summer			
Dragonflies	26	30.5 (± 4.8)	33.72
Butterflies	17	67 (± 59.58)	34.77
Plants	114	161.12 (± 21.72)	167.97
Late summer			
Dragonflies	25	34 (± 10.17)	34.45
Butterflies	29	45.67 (± 14.85)	45.04
Plants	114	161.12 (± 21.72)	167.97
Grasshoppers	46	52.25 (± 5.17)	57.71

3.3.1 Species richness response to zone

Dragonfly ($\chi^2 = 95.74$, $p < 0.001$, $df = 4$) and butterfly ($\chi^2 = 76.90$, $p < 0.001$, $df = 4$) species richness differed significantly between the ecotone zones in the early summer. Dragonfly species richness was highest in the deep marsh and shallow marsh zones, and lowest in the grassland and open water zones (Figure 3.2A). Butterfly species richness was highest in the grassland zone, with no species in the open water zone, one species in the marsh zones and few species in the wet meadow zones (Figure 3.2B).

Dragonfly ($\chi^2 = 38.52$, $p < 0.001$, $df = 4$), butterfly ($\chi^2 = 263.47$, $p < 0.001$, $df = 4$), and grasshopper ($\chi^2 = 128.82$, $p < 0.001$, $df = 4$) species richness differed significantly between the ecotone zones in late summer. Dragonfly species richness was highest in the deep and shallow marsh zones, and lowest in the grassland and open water zones (Figure 3.3A). Butterfly species richness was highest in the grassland zone, with no species in the open water zone and few species in the marsh and wet meadow zones (Figure 3.3B). Adult grasshoppers were only recorded in the grassland zone.

Plant species richness differed significantly between zones ($\chi^2 = 125.62$ $p < 0.001$, $df = 4$). Species richness was highest in the drier zones (grassland, wet meadow, and shallow marsh zones), decreasing in the deep marsh zone, and lowest in the open water zone (Figure 3.3C).

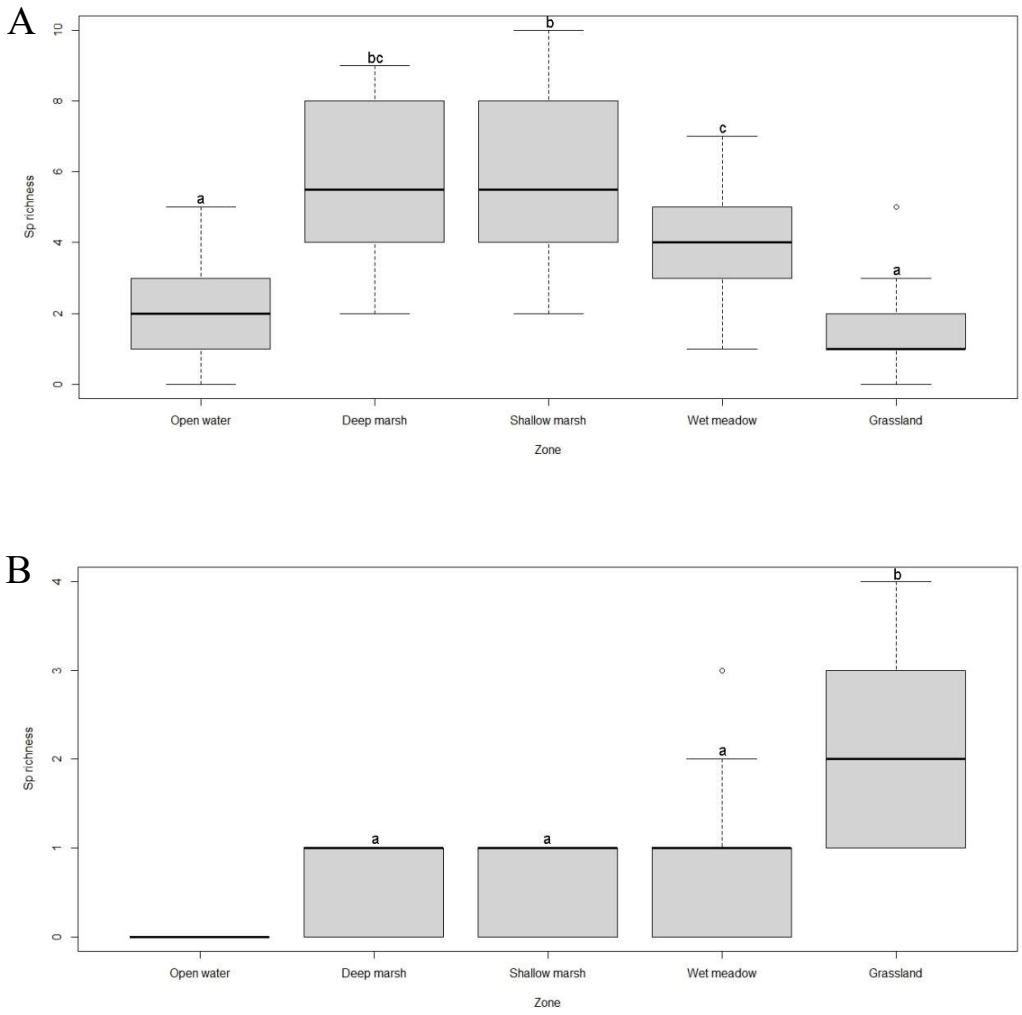

Figure 3.2. Boxplots showing species richness between the different zones (open water, deep marsh, shallow marsh, wet meadow, and grassland) of A: dragonflies and B: butterflies in early summer. Letters above each box indicate significantly different means determined by Tukey post hoc tests where $p < 0.05$

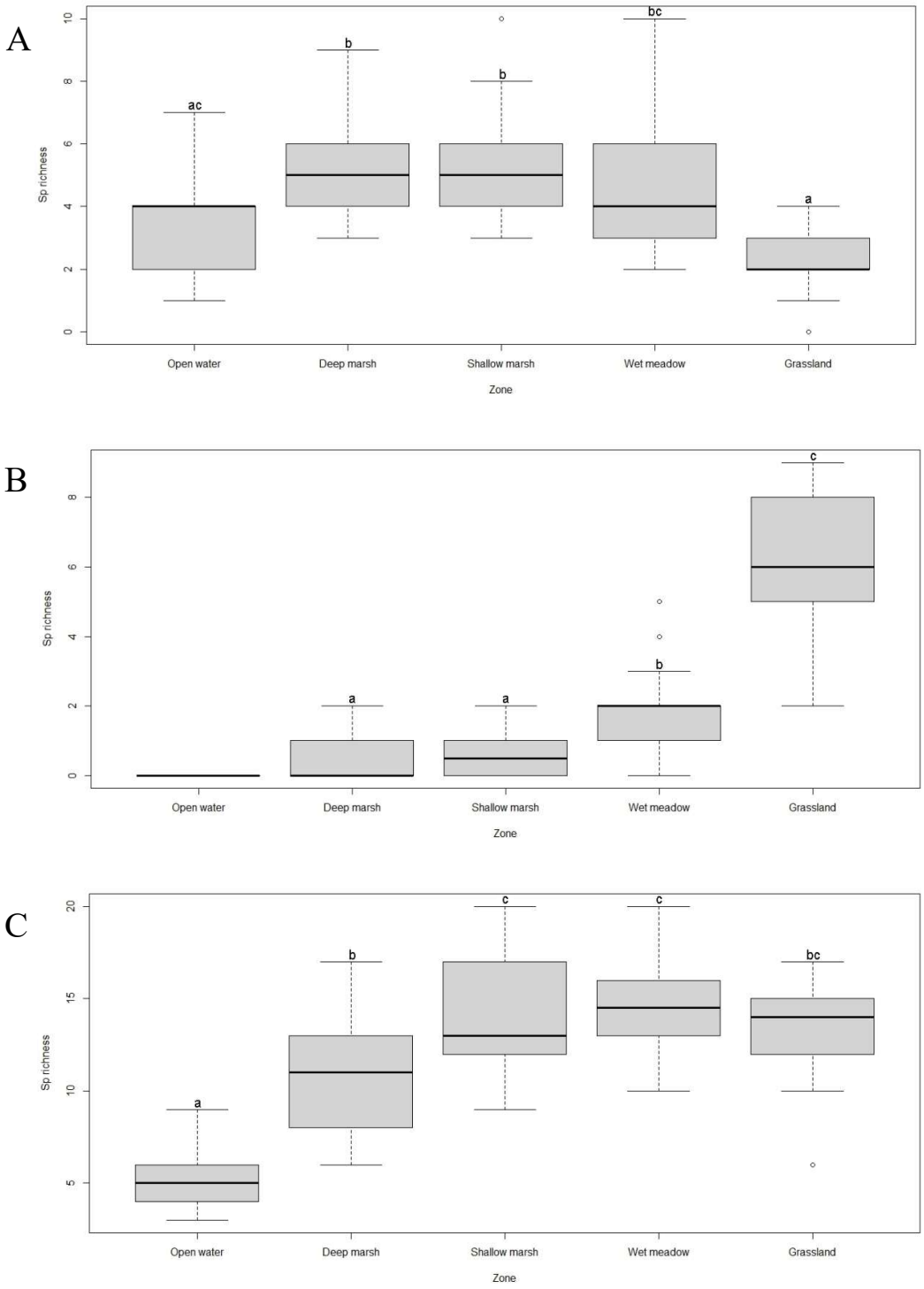

Figure 3.3. Boxplots showing species richness between the different zones (open water, deep marsh, shallow marsh, wet meadow, and grassland) of A: dragonflies and B: butterflies in late summer and C: plants. Letters above each box indicate significantly different means determined by Tukey post hoc tests where $p < 0.05$

3.3.2 Species composition among zones

Dragonfly (F = 4.65, p = 0.002, df = 4) and butterfly (F = 4.09, p = 0.011, df = 3) species composition differed significantly among zones in early summer. Dragonfly (F = 5.18, p < 0.001, df = 4) and butterfly (F = 4.39, p = 0.005, df = 3) species composition differed significantly among zones in late summer. Plant species composition differed significantly among zones (F = 4.67, p = 0.001, df = 4).

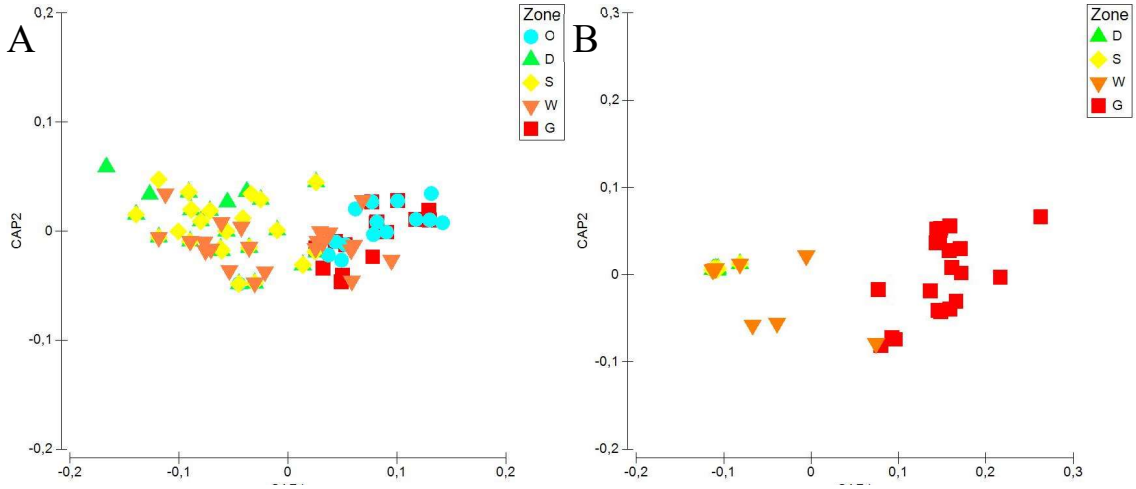

Figure 3.4. Canonical analysis of principal coordinates (CAP) for A: dragonfly and B: butterfly species composition structure in early summer between the ecotone zones. Abbreviations: O – open water, D – deep marsh, S – shallow marsh, W – wet meadow, G – grassland

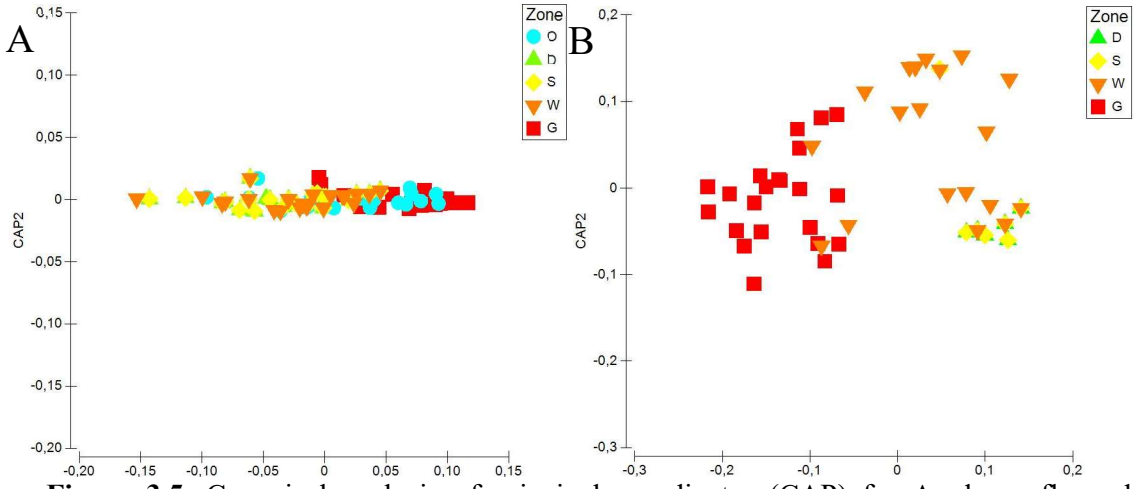

Figure 3.5. Canonical analysis of principal coordinates (CAP) for A: dragonfly and B: butterfly species composition structure in late summer between the ecotone zones. Abbreviations: O – open water, D – deep marsh, S – shallow marsh, W – wet meadow, G – grassland

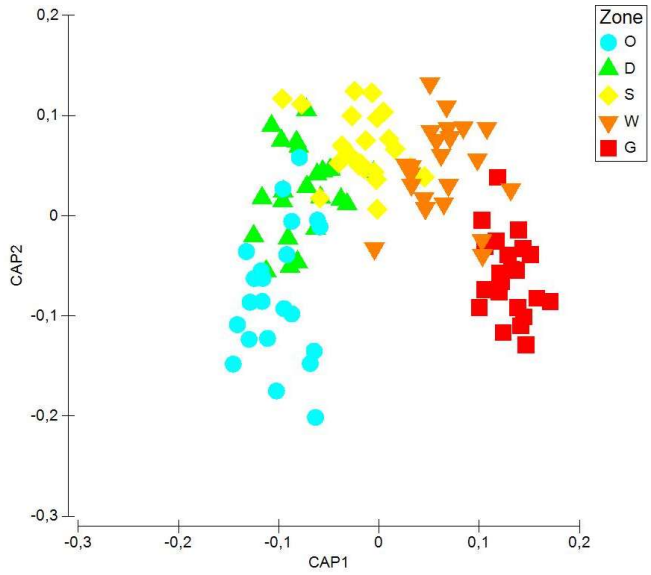

Figure 3.6. Canonical analysis of principal coordinates (CAP) for plant species composition among ecotone zones. Abbreviations: O – open water, D – deep marsh, S – shallow marsh, W – wet meadow, G – grassland

PERMANOVA pairwise tests indicated that dragonfly species composition in early summer was significantly different among all the zones, with two exceptions: there were no significant differences between shallow marsh and wet meadow and open water and grassland. Dragonfly species composition late summer showed significant differences between deep marsh all other zones, with the highest significant dissimilarity between deep marsh and shallow marsh. There were also significant differences in species composition between shallow marsh, wet meadow, and grassland zones. Butterfly species composition in early summer differed significantly between the grassland zone and the other zones (deep marsh, shallow marsh, and wet meadow). Butterfly species composition in late summer was only similar between deep marsh and shallow marsh zones and differed significantly among all other zones. Plant species composition differed significantly between all zones, with the exception of no significant difference between open water and deep marsh zones, and deep marsh and shallow marsh zones.

Table 3.2. PERMANOVA Pseudo-F and pairwise t test results for species composition of dragonflies, butterflies, and plants between the five ecotone zones. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland. (E) – early summer, (L) – late summer.

Zones	Dragonflies (E)	Dragonflies (L)	Butterflies (E)	Butterflies (L)	Plants
All	**4.65*** **	**5.18*** **	**4.09***	**4.39****	**4.67*** **
OW - DM	2.32**	1.86*	-	-	1.34
OW - SM	2.25**	1.87	-	-	1.74***
OW - WM	2*	1.45	-	-	1.88***
OW - GR	0.72	1.42	-	-	2.34***
DM - SM	4.08***	29.61***	2.95	1.49	1.8
DM - WM	1.79*	2.05	1.59	1.94*	2.09**
DM - GR	2.46**	2.99**	2.34***	2.28**	2.54***
SM - WM	1.63	2.35*	1.57	1.76*	1.99**
SM - GR	2.43**	3.03**	2.32***	2.12**	2.77***
WM - GR	2.46**	2.76**	2.03**	2.1**	2.23***

Pseudo-F values are in bold. Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

3.3.3. Beta diversity

Dragonflies had the highest alpha diversity in the deep marsh, shallow marsh and wet meadow zones, a trend seen in both early and late summer. Butterflies had the highest alpha diversity in the grassland zone, which then decreased as the zones became wetter, in both early and late summer for butterflies. Grasshoppers had the highest alpha diversity in the grassland zones, with no adult grasshoppers in any other zone. Plants had the lowest alpha diversity in the open water zone and alpha diversity increased as the zones got drier, with highest alpha diversity in the wet meadow zone.

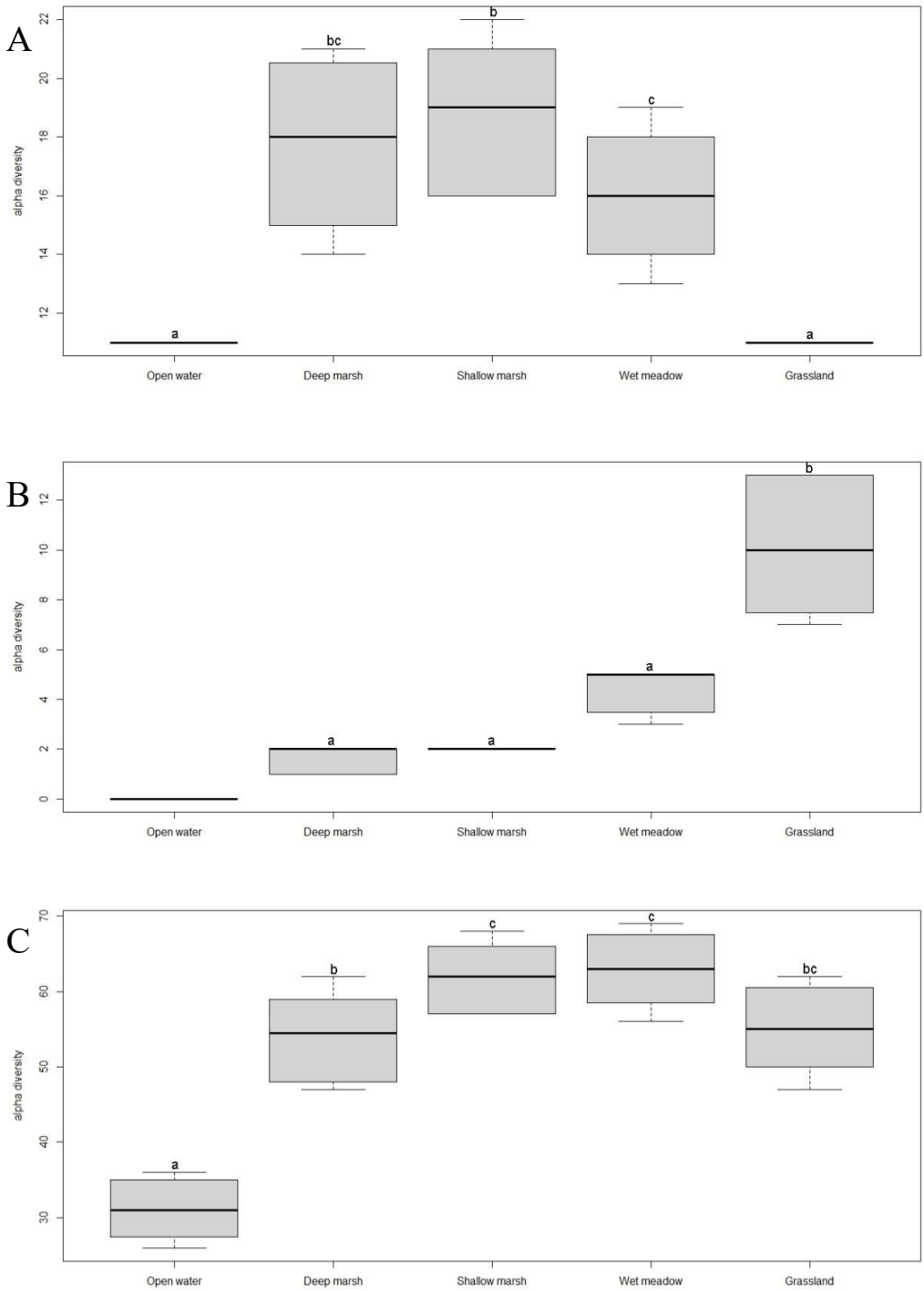

Figure 3.7. Boxplots showing rarefied alpha diversity across ecotone zones for A: dragonflies and B: butterflies in the early summer and C: plants

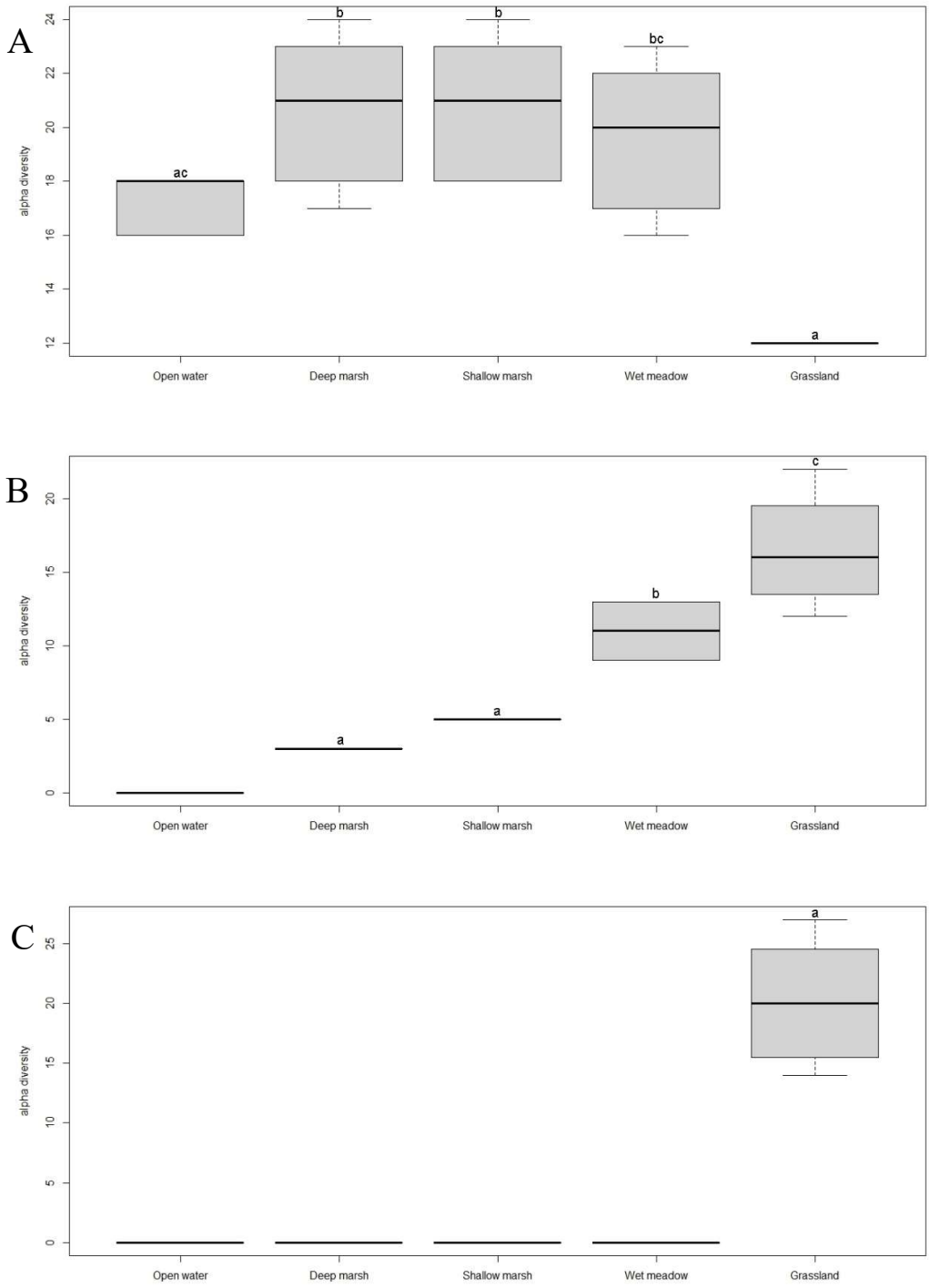

Figure 3.8. Boxplots showing rarefied alpha diversity across ecotone zones for A: dragonflies, B: butterflies and C: grasshoppers in late summer

Species assemblages differed between the ecotone zones. In the early summer, dragonfly beta diversity was driven by differences in species richness, with grassland and marsh zones most distinct from each other, and deep marsh and shallow marsh being least dissimilar (Table 3.3). The same trend was seen late summer, whereby dragonfly beta diversity was driven by differences in species richness with the deep marsh zone being equally highly distinct from open water and wet meadow zones (Table 3.4). The wet meadow and open water zones had the least dissimilarity, even though the zones were far apart.

Table 3.3. Beta diversity between zones for dragonflies in early summer, shown as total beta diversity and its two constituents: replacement beta diversity and richness beta diversity. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland.

Total beta diversity				
	OW	DM	SM	WM
DM	0.77			
SM	0.77	0.04		
WM	0.61	0.47	0.44	
GR	0.34	0.82	0.82	0.69
Beta diversity due to species replacement				
	OW	DM	SM	WM
DM	0			
SM	0	0.04		
WM	0.01	0.06	0.02	
GR	0.16	0.02	0.01	0.01
Beta diversity due to change in species richness				
	OW	DM	SM	WM
DM	0.77			
SM	0.77	0		
WM	0.6	0.41	0.42	
GR	0.18	0.81	0.81	0.68

Table 3.4. Beta diversity between zones for dragonflies in late summer, shown as total beta diversity and its two constituents: replacement beta diversity and richness beta diversity. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland.

Total beta diversity				
	OW	DM	SM	WM
DM	0.65			
SM	0.41	0.42		
WM	0.01	0.65	0.41	
GR	0.09	0.62	0.37	0.10
Beta diversity due to species replacement				
	OW	DM	SM	WM
DM	0.02			
SM	0	0.05		
WM	0	0.02	0	
GR	0	0.03	0.03	0
Beta diversity due to change in species richness				
	OW	DM	SM	WM
DM	0.63			
SM	0.41	0.37		
WM	0.01	0.63	0.41	
GR	0.09	0.59	0.35	0.10

In the early summer, butterfly beta diversity was driven by species replacement, with the exception of the shallow marsh and wet meadow zones, where beta diversity was driven by differences in species richness (Table 3.5). The grassland zone was equally and highly distinct from open water, deep marsh, and shallow marsh zones. The least dissimilarity was between deep marsh and shallow marsh zones. There was a different trend late summer when butterflies were more speciose as beta diversity was driven solely by a difference in species richness (Table 3.6). The grassland and open water zones were most distinct from each other, while least dissimilarity was between deep marsh and shallow marsh zones.

Table 3.5. Beta diversity between zones for butterflies in early summer, shown as total beta diversity and its two constituents: replacement beta diversity and richness beta diversity. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland.

Total beta diversity				
	OW	DM	SM	WM
DM	1			
SM	1	0.13		
WM	1	0.29	0.21	
GR	1	1	1	0.94
Beta diversity due to species replacement				
	OW	DM	SM	WM
DM	0			
SM	0	0		
WM	0	0.26	0.06	
GR	0	0.65	0.59	0.57
Beta diversity due to change in species richness				
	OW	DM	SM	WM
DM	1			
SM	1	0.13		
WM	1	0.03	0.15	
GR	1	0.35	0.41	0.36

Table 3.6. Beta diversity between zones for butterflies in late summer, shown as total beta diversity and its two constituents: replacement beta diversity and richness beta diversity. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland.

Total beta diversity				
	OW	DM	SM	WM
DM	1			
SM	1	0.13		
WM	1	0.61	0.57	
GR	1	0.90	0.90	0.66
Beta diversity due to species replacement				
	OW	DM	SM	WM
DM	0			
SM	0	0		
WM	0	0.24	0.05	
GR	0	0.10	0.11	0.09
Beta diversity due to change in species richness				
	OW	DM	SM	WM
DM	1			
SM	1	0.13		
WM	1	0.59	0.52	
GR	1	0.80	0.77	0.57

Grasshopper adults were present only late summer, and only in the grassland zone. Only nymphs were recorded in the moist and wet zones. Due to grasshoppers being in only one zone, beta diversity = 1 between the grassland zone and all other zones and was attributed to differences in species richness.

Plant beta diversity was driven solely by species replacement, meaning different plant assemblages per zone. Grassland and open water zones were most distinct from each other, while deep marsh and shallow marsh zones were similar (Table 3.7).

Table 3.7. Beta diversity among zones for plants, shown as total beta diversity and its two constituents: replacement beta diversity and richness beta diversity. Abbreviations: OW – open water, DM – deep marsh, SM – shallow marsh, WM – wet meadow, GR – grassland.

Total beta diversity				
	OW	DM	SM	WM
DM	0.52			
SM	0.72	0.48		
WM	0.88	0.77	0.53	
GR	0.97	0.93	0.80	0.59
Beta diversity due to species replacement				
	OW	DM	SM	WM
DM	0.29			
SM	0.50	0.45		
WM	0.66	0.72	0.51	
GR	0.76	0.87	0.76	0.57
Beta diversity due to change in species richness				
	OW	DM	SM	WM
DM	0.23			
SM	0.22	0.03		
WM	0.22	0.05	0.03	
GR	0.21	0.06	0.04	0.02

Each zone was investigated as its own unit across the 22 sites to determine whether the changes within zones are due to species replacement or differences in species richness, to investigate whether each pond was unique (with its own suite of species with preferences for different zones) or whether the ponds consisted of similar species with potential movement between zones. In early summer, dragonfly beta diversity was driven solely by species replacement in all the zones (Appendix D). The grassland and open water zones were distinct from each other and the deep marsh, shallow marsh, and wet meadow zones. The deep marsh, shallow marsh, wet meadow zones were similar to each other, also seen late summer, where dragonfly beta diversity was solely driven by species replacement in all zones, with the open water zone most distinct from all other zones (Appendix E). Deep marsh, shallow marsh, wet meadow, and grassland all had low dissimilarity.

In early summer, butterfly beta diversity in grassland and wet meadow zones was driven by species turnover, whereas butterfly beta diversity in the deep marsh and shallow marsh zones was driven by species richness (Appendix F). Only shallow marsh and wet meadow zones were distinct from other zones. In late summer, there was a similar trend for butterfly beta diversity in grassland and wet meadow zones, driven by species turnover. In contrast, butterfly beta diversity in the deep marsh and

shallow marsh zones was driven by species richness (Appendix G). The grassland zone was distinct from all other zones.

In late summer, grasshopper beta diversity in grassland was driven mostly by species replacement rather than species richness according to the mean values (Appendix H), but the boxplot suggests too much variation to accurately determine whether beta diversity in the grassland zone was due only to species replacement.

Plant beta diversity was driven solely by species replacement in all zones (Appendix I). The open water and grassland zones were distinct from all other zones, whereas deep marsh, shallow marsh and wet meadow had low dissimilarity.

3.3.4 Factors influencing species richness and composition

Wet-associated and dry-associated species were chosen to investigate the effects of environmental gradients on species specifically associated with open water, deep marsh, or shallow marsh zones (aquatic species), and species associated with the grassland zone (terrestrial species). In early summer, 12 aquatic species and 12 terrestrial species were identified. In late summer, 10 aquatic species and 45 terrestrial species were identified.

Of the 13 environmental variables tested for multicollinearity, 10 were used for species richness and composition patterns early summer: soil type, erosion, pond size, pH, water temperature, electrical conductivity, water depth, dissolved oxygen, % plant cover, and average plant height. Nine variables were used to describe species richness and composition patterns in the late summer: soil type, erosion, pond size, pH, water temperature, water depth, dissolved oxygen, % plant cover, and average plant height.

Dragonfly, butterfly, and plant species richness did not significantly respond to any of early or late summer variables. Grasshopper species richness showed a significantly negative response to increasing pond size ($z = (-)2.05$, $p = 0.034$) late summer. The species richness of the aquatic species showed a positive response to erosion early and late summer ($z = (+)2.02$, $p = 0.044$; $z = (+)3.22$, $p = 0.001$) and species richness of terrestrial species showed a negative response to increasing pond size ($z = (-)2.07$, $p = 0.039$) late summer.

Of the 10 environmental variables measured early summer, only six were selected by distance-based modelling (DistLM) as significant: pH, erosion, water temperature, soil type, average plant height, and electrical conductivity (Table 3.8). For dragonfly species composition, 9% of the variation was explained by pH. Variation in butterfly species composition was explained by erosion (17.8%) and

water temperature (11.3%). Plant variation early summer was explained by erosion (21.4%) and pH (8.5%), while aquatic species composition was explained by erosion (21.1%), soil type (11.7%), and average plant height (9.3%). Variation in terrestrial plant species composition was explained by erosion (17.4%) and electrical conductivity (8.5%).

Of the nine late summer environmental variables, only four were selected by distance-based modelling (DistLM) as significant: erosion, pH, water temperature, and average plant height (Table 3.9). Erosion explained 8.5% of variation in dragonfly species composition and 19.1% of the variation in plant species composition. pH explained 10.4% of variation in butterfly species composition and 9.3% variation in grasshopper species composition. Variation in aquatic species was explained by erosion (21.8%), water temperature (13.9%) and average plant height (8.1%), whereas variation in terrestrial species composition was explained by erosion (12.7%) and pH (8.7%).

Table 3.8. Environmental variables most descriptive of species composition from distance-based linear modelling (DistLM) in early summer.

Taxon	Environmental variable	F	Variation explained (%)	Cumulative variation explained (%)
Dragonflies	pH	1.98*	9.01	9.01
Butterflies	Erosion	4.23**	17.81	17.81
	Water temperature	3.12*	11.29	29.11
Plants	Erosion	4.72***	21.36	21.36
	pH	2.91***	8.5	29.87
Aquatic species	Erosion	5.06**	21.11	21.11
	Soil	3.28*	11.73	32.84
	Plant height	3.17*	9.3	42.14
Terrestrial species	Erosion	3.48**	17.39	17.39
	Electrical conductivity	2.83*	8.46	25.85

Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

Table 3.9. Environmental variables most descriptive of species composition from distance-based linear modelling (DistLM) in late summer.

Taxon	Environmental variable	F	Variation explained (%)	Cumulative variation explained (%)
Dragonflies	Erosion	1.75*	8.05	8.05
Butterflies	pH	2.32*	10.39	10.39
Grasshoppers	pH	2.06*	9.33	9.33
Plants	Erosion	4.72***	19.12	19.12
Aquatic species	Erosion	5.03**	21.82	21.82
	Water temperature	3.92*	13.92	35.74
	Plant height	3.25*	8.14	43.88
Terrestrial species	Erosion	2.44*	12.71	12.71
	pH	2.54*	8.67	21.38

Significance levels *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$

3.4. Discussion

Each taxon had its own pattern of species richness and diversity across the zones of the ecotones. The open water zone unsurprisingly only supported dragonflies and plants, and in low species numbers over both seasons. The low dragonfly species in the open water zone was the result of lack of perching vegetation and were largely foragers or they were searching for mates (De Marco Jr et al. 2005). Patrolling as opposed to perching dragonflies, spend less time at the water surface and more likely to span the entire ecotone (Corbet 1962; De Marco Jr et al. 2005; Bried and Ervin 2006), which was the case here. In turn, although dragonflies did not significantly respond to any environmental gradient in either season, their species richness and alpha diversity was highest in both the deep and shallow marsh zones, attributed to vegetation structure, suitable for feeding, breeding, and resting (Samways and Simaika 2016; Valente-Neto et al. 2016; Deacon et al. 2018; Johansson et al. 2019; Briggs et al. 2019). Bried and Ervin (2006) found that mature males tend to dominate the area close to the water while immature females dominated farther from the water. This indicates that the wetland ecotone is the ideal habitat for breeding, also reported by Cunningham-Minnick et al. (2019), which suggests the species richness and diversity peak in the marsh zone here may be due in particular to breeding preferences, as shown here by their response to pH early summer and given that adults can select suitable larvae habitats based on chemistry as well as vegetation (Kietzka et al. 2017). However, a positive response of dragonfly species composition to erosion in late summer was unexpected, and

possibly due to eroded sites having higher water velocity, with additional microhabitats, especially riffles.

Dragonfly species richness was similar but composition very different in deep marsh vs. shallow marsh zones, especially evident early summer when beta diversity was driven by species turnover. Furthermore, there was also dragonfly compositional differences among the marsh zones on different ponds.

Four dragonfly species (*Lestes virgatus*, *Chlorolestes fasciatus*, *Pseudagrion caffrum*, *Pseudogrion kersteni*) were associated with the deep and shallow marsh zones in early summer, but only two species (*Allocnemis leucosticta*, *Chlorolestes fasciatus*) in late summer. Being perching zygopterans, they are closely associated with the water surface to many life functions, although there is some species succession with season.

When zones were analysed as a unit across all 22 ponds, I found that total beta diversity of adult dragonflies between the 22 ponds was driven by species replacement in the case of all zones, meaning that each pond supported its own set of species in each zone. This is important for conservation management of the entire pondscape, with each pond supporting a slightly different species set due to differing microhabitats. This supports the narrative that it is better to conserve many smaller ponds with differing microhabitats and species than one large pond with fewer microhabitats with only habitat generalist species (Oertli et al. 2002; Hill et al. 2018). This is true for the range of pond sizes and types investigated for this study.

Butterflies were scarce early summer, and more diverse and abundant late summer, in keeping with known peak flight periods and abundant nectar resources in this region (Pryke and Samways 2003; Woodhall 2005). They were entirely absent over both seasons from the open water zone and scarce in both deep and shallow marsh zones, and where nectar resources were low. Butterfly species composition was similar in both deep and shallow marsh zones, with beta diversity driven by differences in species richness. There were a few species associated with the marshy area but had no preference for deep vs. shallow marsh zones, and between which they moved freely. The marsh zone was dominated by *Hyalites rahira rahira* (Woodhall 2005), which also foraged at the drier end of the ecotone.

Butterfly species richness and diversity increased sharply from shallow marsh to wet meadow, especially late summer when grassland-associated species made their appearance. Grassland however, with its abundance of nectar resources and breeding sites, supported by far the most species.

Total beta diversity, involving differences in species richness, was high between grassland, with individuals moving freely across zones.

In early summer, five butterfly species were associated with grassland, but by late summer, there were five additional species, among them arrival of migratory species (*Belenois aurota*, *Catopsilia florella*), as well as two *Papilio* spp. and a *Junonia* sp., widespread and attracted to moist areas (Woodhall 2005).

In early summer, butterfly species composition increased in response to erosion, and water temperature and pH. The association with erosion is via the flowering of alien *Verbena bonariensis* in eroded areas (Pryke and Samways 2003). The positive response to higher water temperature is likely a response to higher ambient temperatures as hotter, sunnier days stimulates greater activity. Similarly, increased butterfly species composition with water pH is likely via positive vegetation responses.

In both seasons, beta diversity in both deep and shallow marsh zones across the 22 wetlands was driven by species richness, meaning that marsh-associated butterfly species move freely across the pondscape. This may be due to differing quality and presence of host plants between the marsh zones of the pondscape, as the marsh zones offer a different set of plants to that in the wet meadow and grassland zones. In contrast, beta diversity in wet meadow and grassland zones was driven by species replacement, suggesting that each wetland supports a specific set of grassland-associated butterfly species. From a conservation perspective this means that the entire pondscape with its many microhabitats and linkages, would protect the most diversity.

All grasshoppers were largely confined to the grassland zone, with possibly a small amount of undetected spillover into wet meadow, indicating that in this geographical region at least, ponds contribute little to support grasshopper diversity. However, and perhaps importantly unquantified observations here indicated that the wetter area are important for nymphs early season. This may be important for grasshopper survival, especially in drought years, when even small ponds help maintain grasshopper diversity, as I found here. The positive response of grasshoppers to water pH, like the butterflies, was likely indirect via the soil and vegetation.

In late summer, grasshopper beta diversity among the 22 grassland zones was driven more by species replacement than species richness, indicating that each wetland supports a slightly different assemblage at the dry end of the ecotone. As with the other taxa, this again supports the contention that it is better to conserve many smaller ponds, with their variety of microhabitats and with good linkages, than one big one (Oertli et al. 2002).

Plant species richness was lowest in open water, consisting mostly of submerged and some emergent or floating macrophytes, especially the ecologically important *Typha capensis*, and the alien invasive *Egeria densa* (Griffiths et al. 2015). Plant species composition in open water was similar to that of the deep marsh zone, but composition then changed greatly with a decrease in free water.

Plant species richness and alpha diversity increased from deep marsh to shallow marsh, although species composition was similar for the two zones, indicating a gradual response to reduced water depth. However, overall, from a botanical point of view, the two zones could be considered as one large marsh zone, with a gradual turnover of species as free water decreases, and across the whole ecotone each zone being driven by species replacement and having its own set of plant species.

Only two plant species were associated with deep marsh and shallow marsh zones, indigenous *Cyperus rupestris* and alien *Nasturtium officinale* (Griffiths et al. 2015). Four plant species were associated with water, deep marsh, and shallow marsh zones (*Persicaria lapathifolia*, *Carex austro-africana*, *Juncus lomatophyllus*, *Cladium mariscus*) (Gerber et al. 2004; Griffiths et al. 2015). In turn, seven plant species were associated with grassland, the highly invasive American bramble, *Rubus cuneifolius* and two indigenous pioneer species (*Paspalum dilatatum* and *Tragopogon dubius*) (van Wyk and Malan 1988). Two grass species (*Eragrostis plana* and *Aristida* sp.) and two flowering shrub species (*Senecio* spp) are disturbance-associated plants, indicating grazing by both indigenous game animals and domestic livestock.

Although plant species richness did not respond to any environmental gradient, plant species composition showed a significant response to erosion and pH early season, and erosion late season. Water quality and quantity, as well as pH, are significant variables determining plant communities (Goslee et al. 1997) are important factors affecting plant communities, which here also leads to changes in grasshopper and butterfly assemblages. Added to these effects is that of erosion which enables alien *V. bonariensis* to colonize and then attract feeding butterflies.

Plant beta diversity in each zone among the 22 sites was driven by species replacement, indicating that each pond had its own set of species per zone, supporting not only its own dragonfly, butterfly, and grasshopper assemblages, and like them, also supporting the concept of a conservation pondscape.

Aquatic species richness and species composition showed a positive response to erosion with species composition also showing a preference for increased plant height and clay soil in both seasons. The dragonfly component contributed most to the positive response by plant height, principally because individuals require vegetation on which to perch, rest and retreat from predators (Valente-Neto et al.

2016; Briggs et al. 2019). The positive response by dragonflies to erosion is largely via alien *N. officinale* providing novel microhabitats in high water velocity areas and attracting riffle-associated dragonfly species.

General terrestrial species richness decreased with increasing pond size, largely driven by grasshoppers, comprising 28 out of the 45 selected terrestrial species. Terrestrial species composition early summer responded to erosion and electrical conductivity, and late season, to erosion and pH. Erosion increased the likelihood of invasion by alien plant species, which in turn attracted certain generalist butterflies feeding on alien plants such as *V. bonariensis*. This may in turn deter other sensitive butterflies which struggle to move through dense alien bramble (*R. cuneifolius*). Yet butterflies and grasshoppers also responded to water conditions regardless of preferences for dryland. As even species associated with the terrestrial end of the ecotone are directly or indirectly influenced by water quality, and they in turn, are living and dying, so making nutrients available for runoff into the water. These functional attributes support the contention that wetland conservation should include a large terrestrial buffer zone in order to maintain wetland quality (Dangerfield et al. 2003; Semlitsch and Bodie 2003; Roe and Georges 2007). Future studies would aim to investigate whether larger ponds require larger buffer zones and whether there are factors aside from width that determine the effectiveness of the buffer.

3.5. Conclusions

Each taxon differed significantly in their local distribution across the zones of the aquatic-terrestrial ecotone. Each zone was characterized by a particular set of plant species, and together with water depth, explained the patterns in dragonflies, butterflies and grasshoppers along the ecotone. Dragonflies peaked in the centre of the ecotone, where vegetation structure created the best habitat for life history functions. Butterflies peaked in the grassland zone where there was high host plant diversity, although some species were characteristic of marsh. Grasshoppers showed high fidelity to grassland zone, and a negative response to water body size, although nymphs occupied the moist areas early season.

Ponds had their own suite of species specific to the various component microhabitats. This supports the contention that conservation of many small ponds is better than focussing on one large pond for protection of high species diversity (Oertli et al. 2002; Hill et al. 2018). Terrestrial species responded strongly to water conditions in both seasons, suggesting that a terrestrial buffer zone is required around a pond to maintain its quality. This emphasizes that the entire ecotone is required for effective

biodiversity conservation, while bearing in mind that each pond had its own particular ecotone characteristics. This means that the entire pondscape, including linkages between ponds, should be considered as the conservation unit encompassing a wide range of microhabitats, and hence heterogeneity, and providing individuals of a wide range of species maximal opportunity for selecting optimal habitats for sustaining effective populations.

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Chapter 4: General conclusions

Wetlands are one of the most highly threatened ecosystems, yet are also among the world's most productive environments, providing valuable goods and services. They also have high levels of biodiversity, making wetlands a top conservation priority (Barbier 1993; Sala et al. 2000; Dudgeon et al. 2006; Sandham et al. 2008). To efficiently protect water sources and conserve biodiversity, we require efficient and reliable monitoring methods, such as surrogate indicators that simplify the data requirements for conservation by focusing on a few species to represent the whole community (Leal et al. 2010).

In chapter 2, I aimed to identify a surrogate or suite of surrogates that can represent wetlands and wetland-associated species assemblages for efficient conservation planning. I show that a multi-taxon approach was best, as cross-taxon congruence was not strong enough to identify a single surrogate group. This was found in several previous studies which aimed to test cross-taxon congruence amongst riparian and aquatic communities (Heino 2010; Kirkman et al. 2011; Rooney and Bayley 2012; Tonkin et al. 2016; Roque et al. 2017; Valente-Neto et al. 2018; Carneiro et al. 2019). However, there has been debate over identifying an optimal cross-taxon congruence threshold (de Morais et al. 2018), as assemblages respond differently to environmental gradients and biotic interactions at fine scales and would seldom have levels of congruence high enough to agree with defined congruence thresholds (Padial et al. 2012). Assemblages having similar responses to the same environmental gradient are recorded as one of the main drivers of cross-taxon congruence (Carneiro et al. 2019). My results in chapter 2 demonstrate little shared responses to environmental gradients between the taxa, which further confirms weak cross-taxon congruence.

Dragonflies are well-known potential environmental and ecological indicators (Samways and Simaika 2016) and are strongly congruent with macroinvertebrates in rivers and ponds (Smith et al. 2007; Briggs et al. 2019). My results in chapter 2 demonstrated moderate correlation values between dragonflies and macroinvertebrates, as well as among macroinvertebrates but not high enough to reach an optimal congruence threshold (Lovell et al. 2007; Heino 2010).

Ecoacoustics is a simple non-invasive method of sampling, able to record a relatively large amount of data over large spatiotemporal. It is useful in the case of frogs, which although cryptic, often broadcast distinctive species-specific songs (Xie et al. 2016, 2018; Köhler et al. 2017). Although this could allow for simple and non-invasive wetland monitoring, my results from chapter 2 demonstrate that frogs alone cannot be used as an indicator taxon, as they are not congruent with other wetland-associated taxa. Although frogs are sensitive to environmental change, they do not respond to the same environmental stressors as other wetland taxa.

Studies on the application of the umbrella index are limited, and only demonstrated with birds and butterflies (Betrus et al. 2005), dragonflies and wetland plants (Bried et al. 2007) and dragonflies and EPT taxa in rivers (Kietzka et al. 2019). The umbrella index was applied to dragonflies and frogs at species level, in their adult stage and to aquatic insects at family level in the larval stage. Chapter 2 showed that the umbrella index successfully identified suites of surrogates to identify wetlands requiring conservation management and planning practices. However, due to the lack of cross-taxon congruence, only same-group surrogate species should be selected. The umbrella index was flexible regardless of life stage and identification level, and maximises median rarity allowing for efficient site selection (Fleishman et al. 2001), suggesting that the umbrella index should continue to be used in conservation planning.

Frog populations are highly dynamic and fluctuate greatly with annual variations in climate (Semlitsch et al. 1996). They occur as metapopulations across the pondscape (Kirkman et al. 2012), which is a similar dispersal pattern to that of highly mobile aquatic insects such as dragonflies, beetles and bugs that fly between ponds on the basis of habitat preference. Future studies could aim to investigate cross-taxon congruence of aquatic and riparian communities at larger spatial scales, as it is likely that congruence would be much more significant at the pondscape level by accounting for metapopulation movement (Oertli et al. 2009). A pondscape is a network of ponds and their surrounding terrestrial matrix and are known to have higher conservation value than one large pond (Hill et al. 2018). Chapter 2 set out to investigate the size of a pondscape, and I found that a minimum of 20 ponds should be included in pondscape for pond diversity conservation and protect most of wetland associated taxa. A network of smaller freshwater habitats (pondscapes) should be incorporated alongside large-scale habitat networks to improve the effectiveness of landscape conservation. Ecological networks of remnant land, set aside to mitigate the effects of agroforestry, are rich in natural and artificial pondscapes, supporting similar biodiversity to that of protected areas (Pryke et al. 2015). Management of ENs should aim to increase connectivity between ponds, ensuring that populations can move freely between microhabitats enabling them to successfully fulfil all life history functions.

Wetlands are transitional zones between standing water and terrestrial environments, forming an ecotone where the local wetland and dryland species and communities overlap. Yet, there is limited knowledge on how organisms utilise this ecotone. Wetland ecotones are generally viewed as overlapping communities, but ecotones can be species-rich and can be considered a core habitat for some semi-aquatic and ecotone species, and thus have conservation priority (Semlitsch and Jensen 2001). In chapter 3, I investigated how species assemblages change over the wetland ecotone, by dividing it into five zones and analysing changes in species richness and diversity per zone.

Dragonflies showed a peak in the intermediate marsh zones, where the response of adult dragonflies to marginal vegetation was most apparent. The vegetation structure and composition of the marsh zone created an ideal habitat for most life history functions, such as perching, breeding, and feeding (Samways and Simaika 2016; Deacon et al. 2018; Briggs et al. 2019). Butterflies are indicators of habitat quality, demonstrating preference for high quality habitats (Pryke and Samways 2003). They peaked in the grassland zone, where there is high host plant diversity, with a few marsh-associated species common throughout the pondscape. Grasshoppers, also an indicator of habitat quality (Bazelet and Samways 2012), showed high fidelity to the grassland zone and a negative response to water body size. Future studies should aim to investigate the function of wetlands in orthopteran life history, as nymphs here occupied moist areas during the early season, indicating that wetlands may be important for early life stages. My study focussed on grasshopper adults, which were confined to the grassland zone. There is also possibly a small amount of undetected spillover in the wet meadow zone, but it is clear that wetlands contribute little to support adult grasshopper diversity. Both grassland insect taxa, butterflies, and grasshoppers, demonstrated a compositional response to water quality (pH), which is likely via positive vegetation responses, but illustrates water influence far along the ecotone, thus wetlands may be bigger than we previously thought.

To investigate the environmental drivers of the species assemblage patterns seen along the ecotone, I grouped aquatic species (plants and dragonflies) and terrestrial species (plants, butterflies, and grasshoppers) to determine the significant environmental factors at either end of the ecotone. Aquatic species showed a positive response to plant height, especially among dragonflies, whose response to vegetation structure is strong, as they require vegetation for perching, resting and retreat from predators (Valente-Neto et al. 2016; Briggs et al. 2019). There was a positive response to water margin erosion, seen in both aquatic and terrestrial species. This is attributed to an increase of alien plants, which in turn attracted generalist species. Terrestrial species showed a significant response to water quality variables (electrical conductivity and pH) even in the dryland zone, which suggests that water quality effects reach farther than typical buffer zones. Studies have found the accepted 30-60 m terrestrial buffer zone to be inadequate for many wetland-associated species, suggesting a buffer zone of a few hundred metres to be more appropriate (Semlitsch and Bodie 2003; Roe and Georges 2007). My results in chapter 3 demonstrate that a wetland and its associated terrestrial buffer zone may be relatively large because terrestrial species sampled here, are influenced by water quality variables. Wetland conservation and management practices may wish to focus on increasing the area of water source protection to include a larger amount of its terrestrial matrix.

To define variation in biodiversity seen among zones, I used beta diversity, a measure that portrays changes in species composition between local assemblages and can reflect differences in assemblage

composition between zones (Whittaker 1960; Anderson et al. 2011; Johansson et al. 2019). Beta diversity is based on compositional dissimilarity due to differences in species richness (i.e. a nested assemblage) or dissimilarity due to spatial turnover derived from replacement of species (Baselga et al. 2007; Johansson et al. 2019).

For dragonflies, butterflies and grasshoppers, beta diversity within each site was mostly attributed to differences in species richness. This means that most species moved freely between the zones with no specific sets of species to describe zones. Interestingly, dragonfly beta diversity was driven most by species turnover between deep marsh and shallow marsh zones, suggesting that there may be a different set of species occupying each marsh zone. The ecotone of wetlands is important for dragonfly biodiversity because marginal vegetation of differing water depths hosts different species due to microhabitat preferences. The maintenance of an ecotone ensures the highest vegetation structural diversity for microhabitat preferences, ensuring dragonfly biodiversity. Beta diversity of plants within each site was driven by species turnover, and each zone was characterised by a specific set of species driven by water depth.

To determine the changes in species assemblages in each zone, I investigated the drivers of beta diversity between the zones across the 22 ponds. Adult dragonfly beta diversity was driven by species replacement in all zones, which means that each pond supported its own set of species, driven by microhabitat complexities. Marsh-associated butterflies were similar across the pondscape, but in contrast, grassland butterflies were different at each pond. Grasshopper beta diversity seemed to be driven equally by differences in species richness and species turnover. Plant species were different at each pond and in each zone.

The combined responses in chapter 3 agree that it is better to conserve many smaller ponds with differing microhabitats and species than one large pond with fewer microhabitats with only habitat generalist species (Oertli et al. 2002; Hill et al. 2018). The pondscape encompasses a wide range of microhabitats, supporting habitat heterogeneity and individuals of a wide range of aquatic, semi-aquatic, ecotone and terrestrial fauna and flora. The entire pondscape, including linkages between ponds, should be considered for conservation and management planning.

The economic and conservationist interests of freshwater sources are often in opposition and only by chance is some freshwater biodiversity protected through legislation protecting water resources. The development of ecological networks (ENs) in South African agroforestry plantations have played a major role in conserving aquatic insects (Kietzka et al. 2015; Pryke et al. 2015), and it is crucial that we continue to make progress in the conservation interests of freshwater biodiversity by conserving the full complement of natural water bodies (Samways 2020). This includes the maintenance of river

systems to ensure the maintenance of healthy deposition pools and recharging still water bodies and the construction and maintenance of artificial ponds, which add value to the pondscape (Samways et al. 2020).

The combined results of chapter 2 and 3 highlight the value of conserving the entire pondscape, including a minimum of 20 ponds to ensure wetland biodiversity conservation. This is true for the range of pond types and sizes sampled for this study. This requires the management planning and maintenance of the ponds and their surrounding terrestrial zones, including the linkages between them. It is evident that there is high value in including ponds and wetland patches in conservation corridors. In doing so, aquatic taxa can be conserved alongside terrestrial taxa with few additional management interventions. It is also highly evident that ensuring not only the pond, but the terrestrial matrix around the pond is also of high quality adds exceptionally high value to conserved portions of land.

For the monitoring and management plans of freshwater biodiversity in pondscapes in ENs, I recommend the use of a multi-taxon approach when using insect bioindicators and applying the umbrella index for site prioritisation provided same-group species are used.

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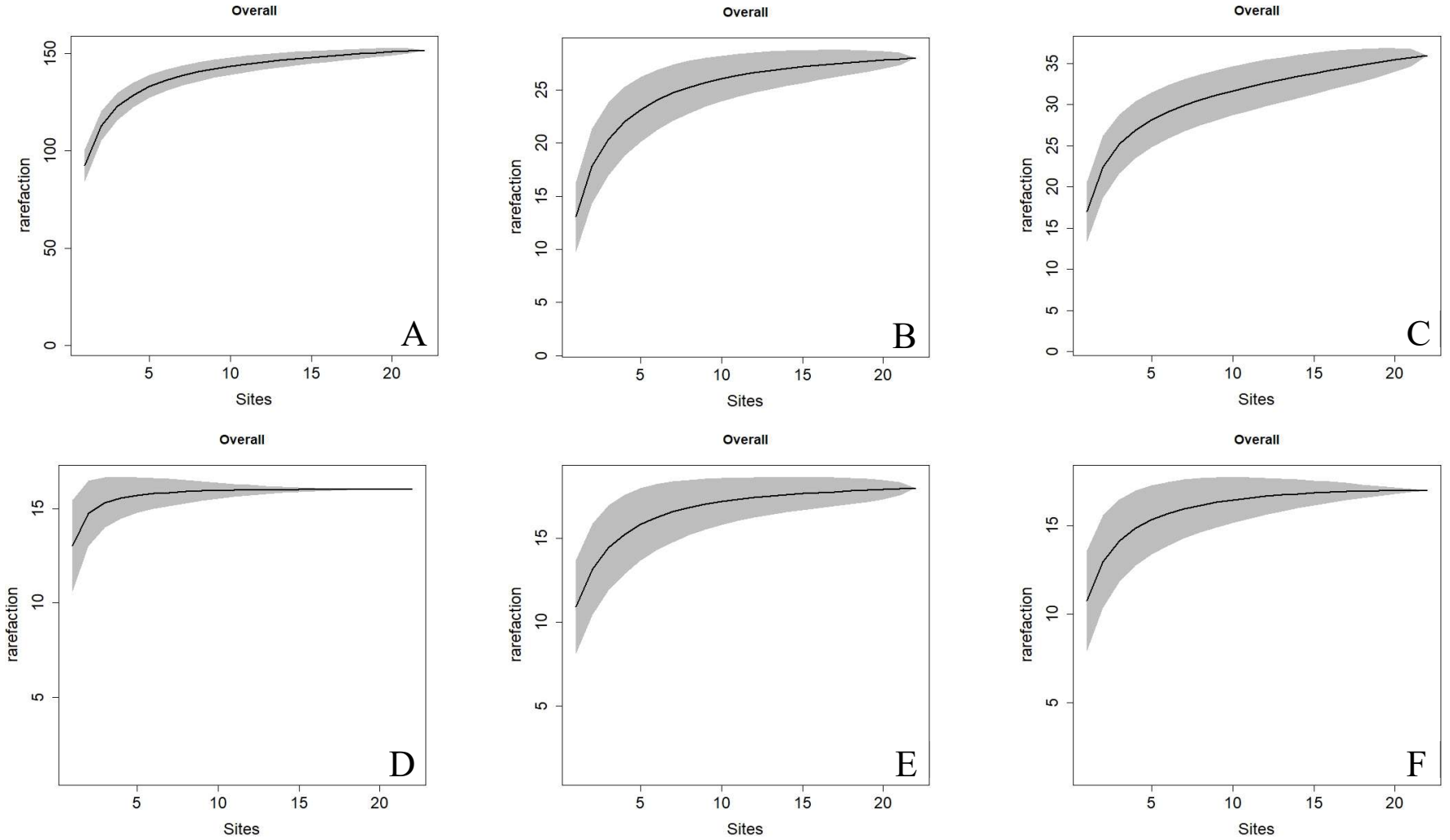
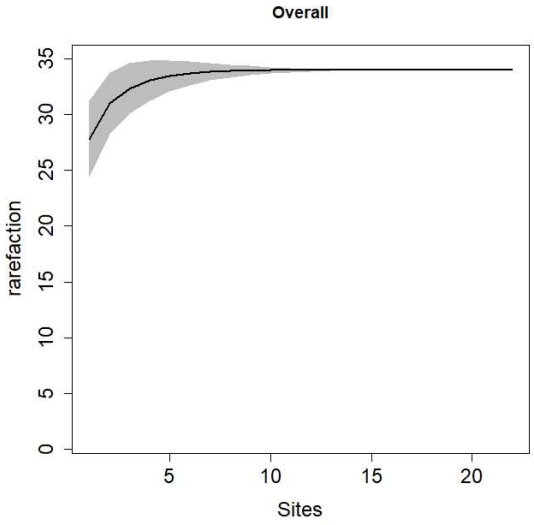
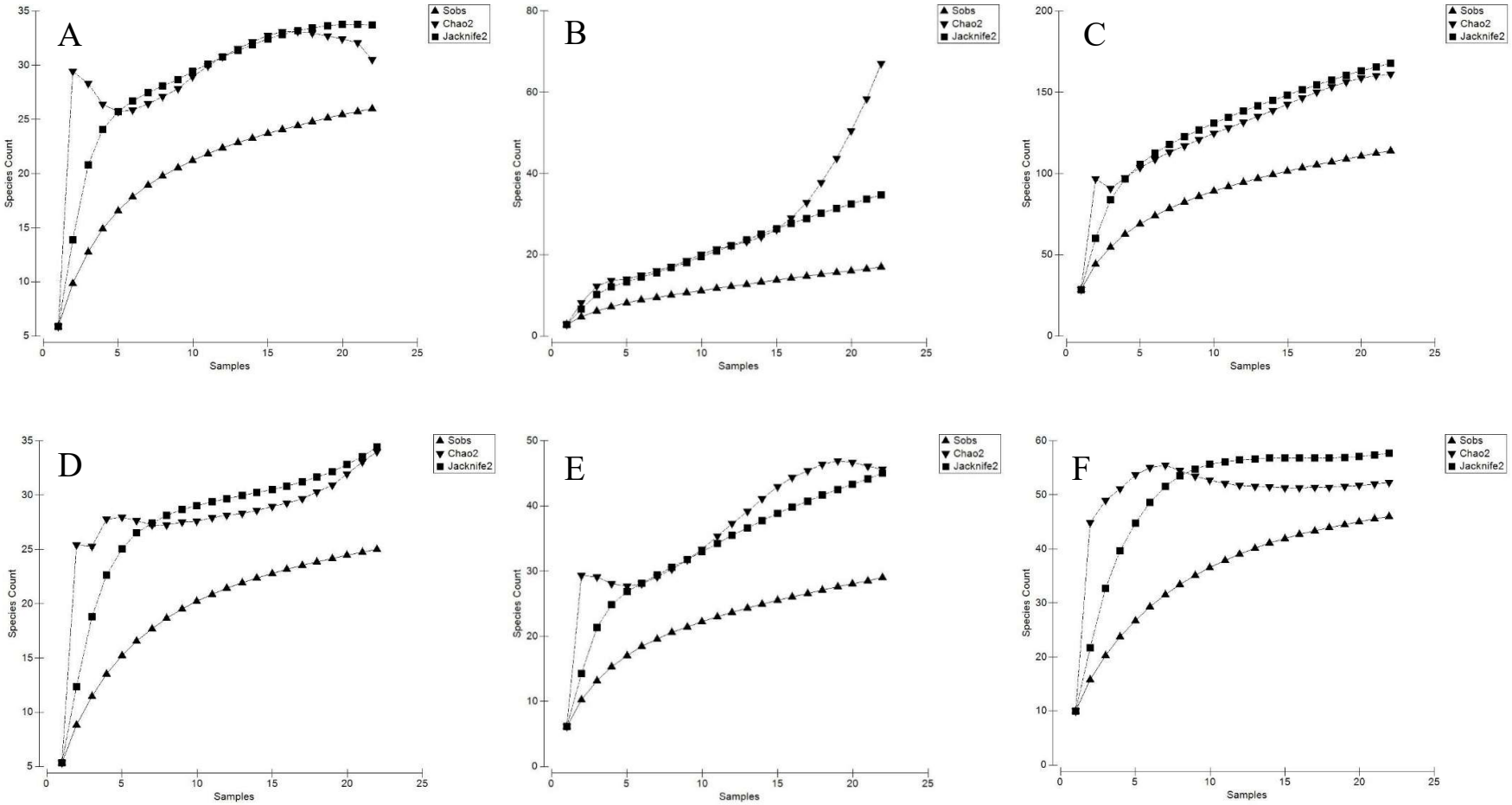
Appendices



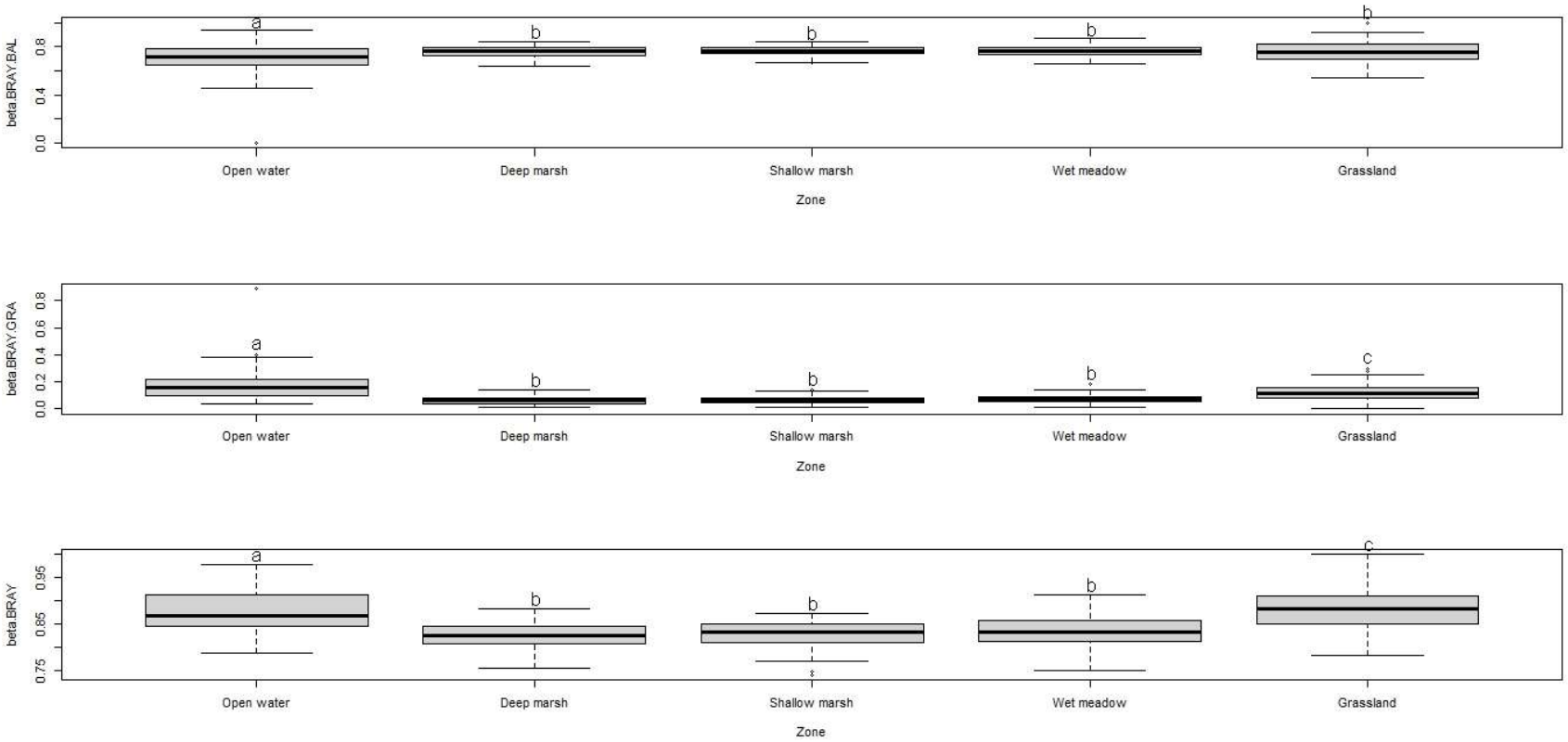
Appendix A. Abundance based rarefaction curves for A: all taxa combined, B: dragonfly adults, C: beetles, D: bugs, E: dragonfly larvae and F: frogs



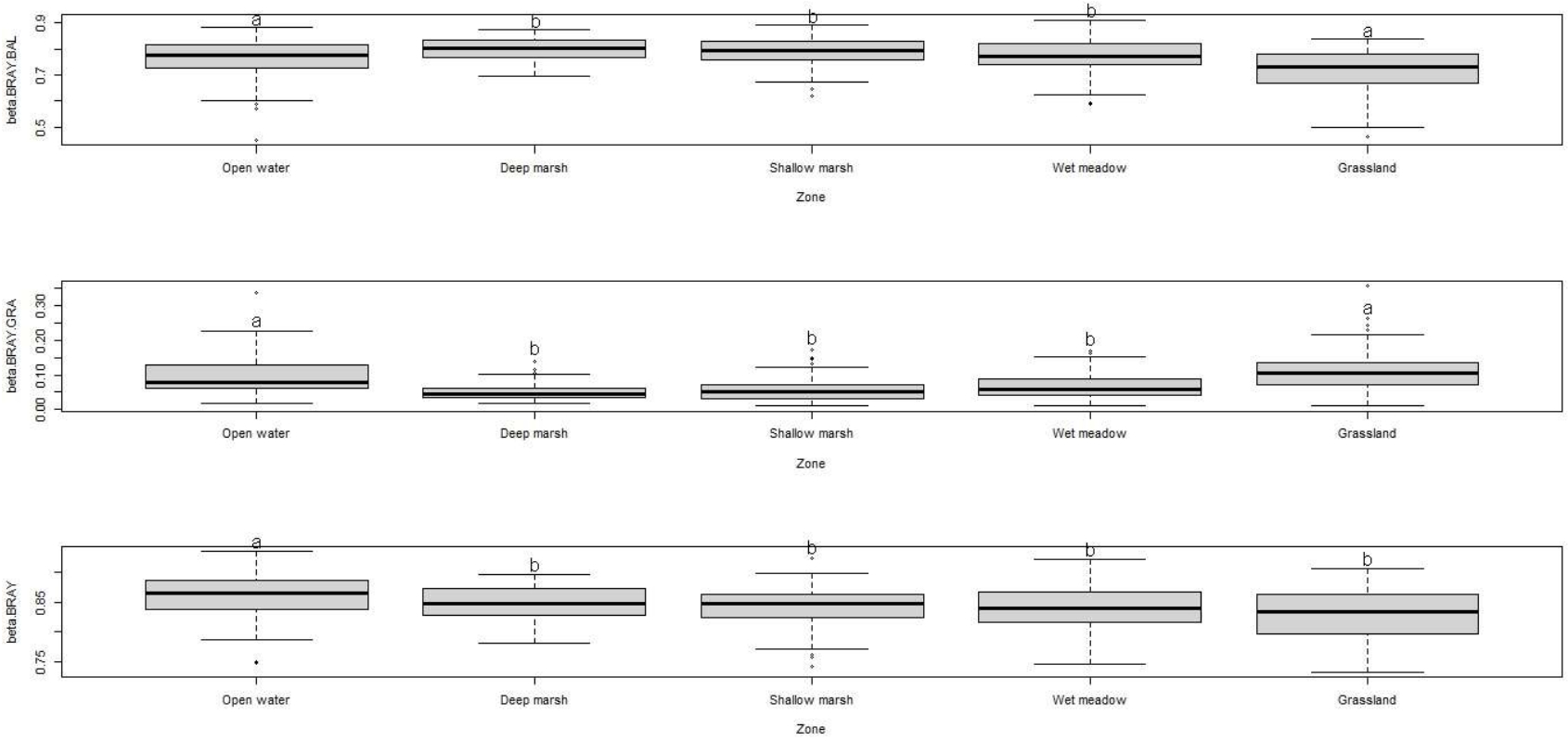
Appendix B. Abundance based rarefaction curves for plants



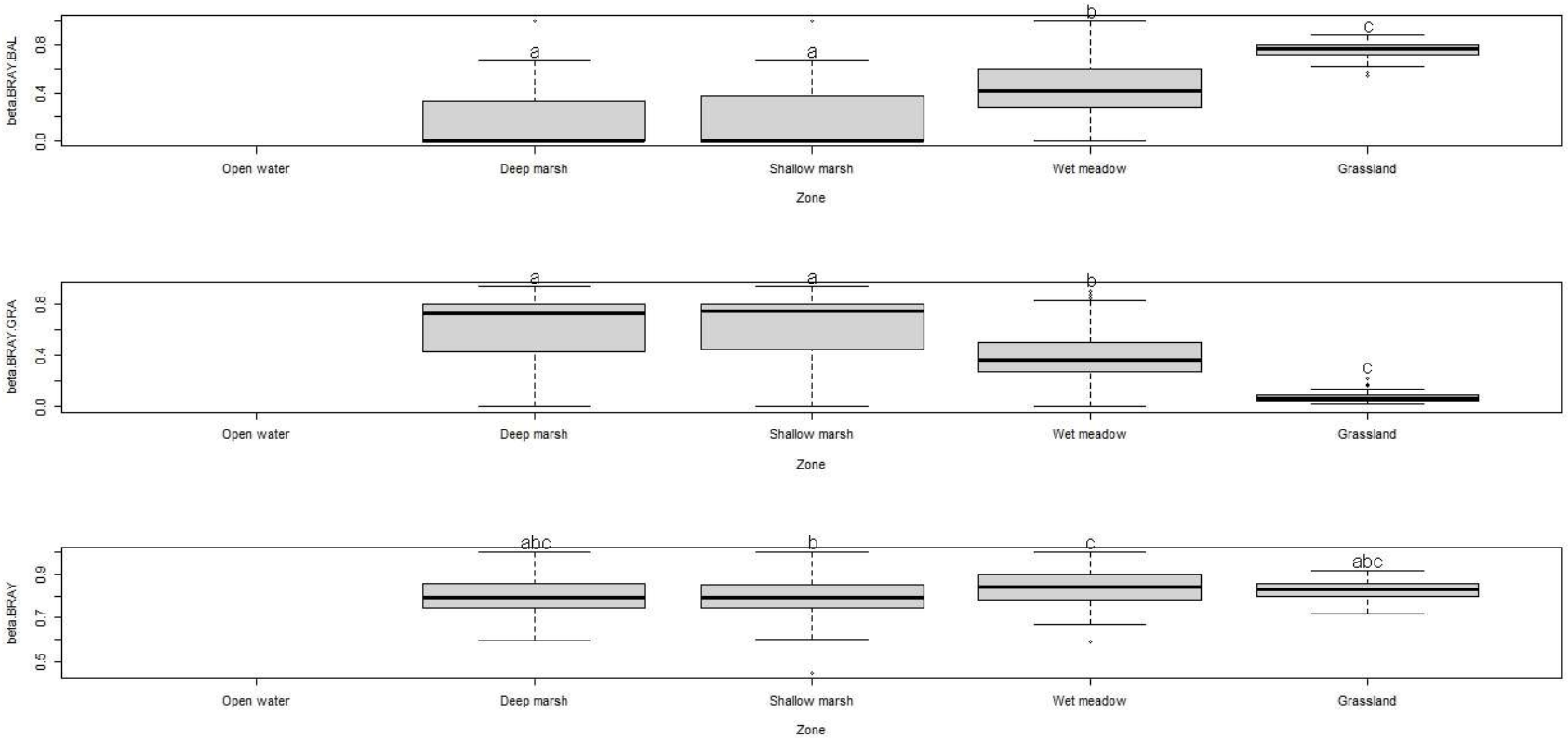
Appendix C. Species accumulation curves of A: dragonflies and B: butterflies in the early summer, C: plants, and D: dragonflies, E: butterflies and F: grasshoppers in the late summer



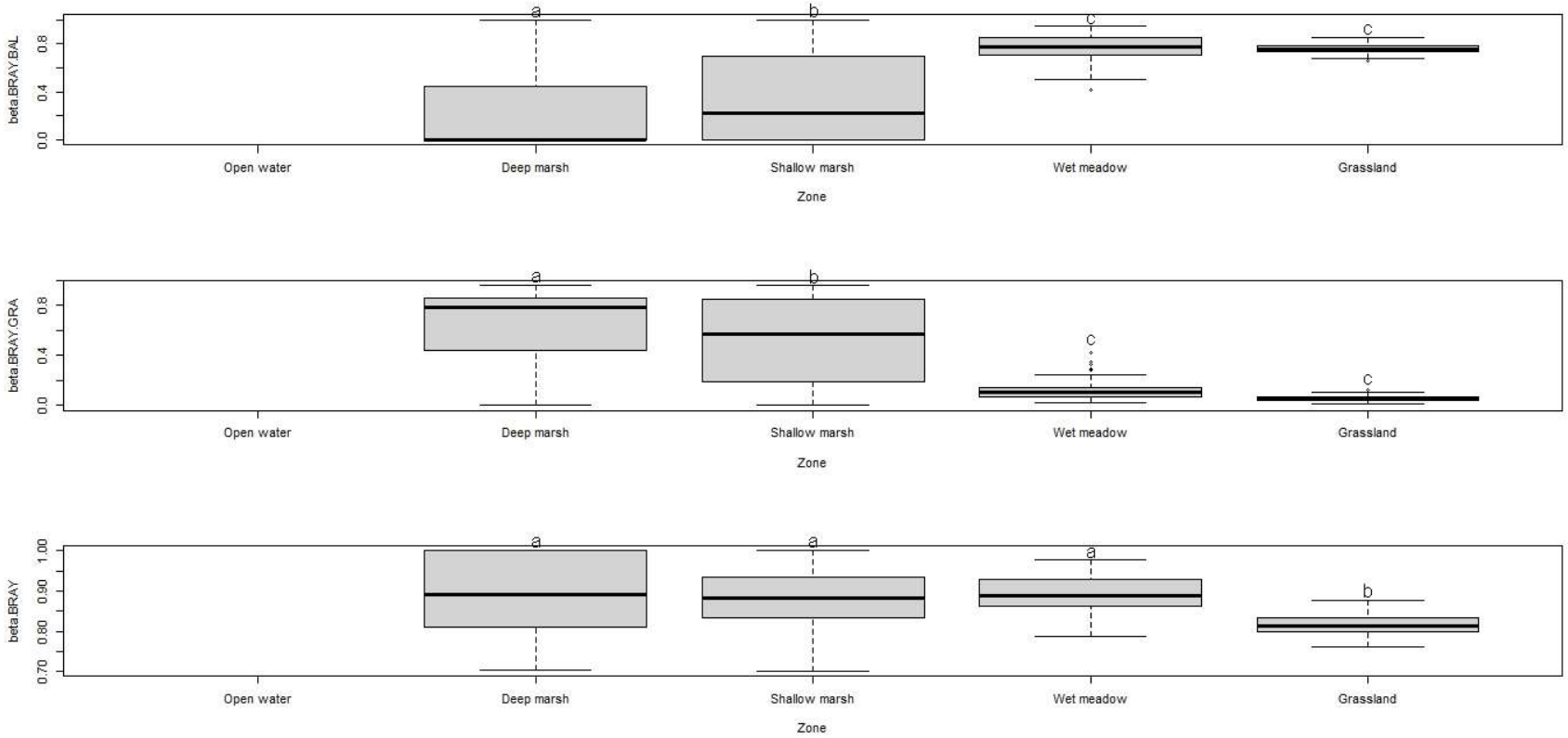
Appendix D. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for dragonflies in the early summer. Different letters above boxes indicate significantly different means.



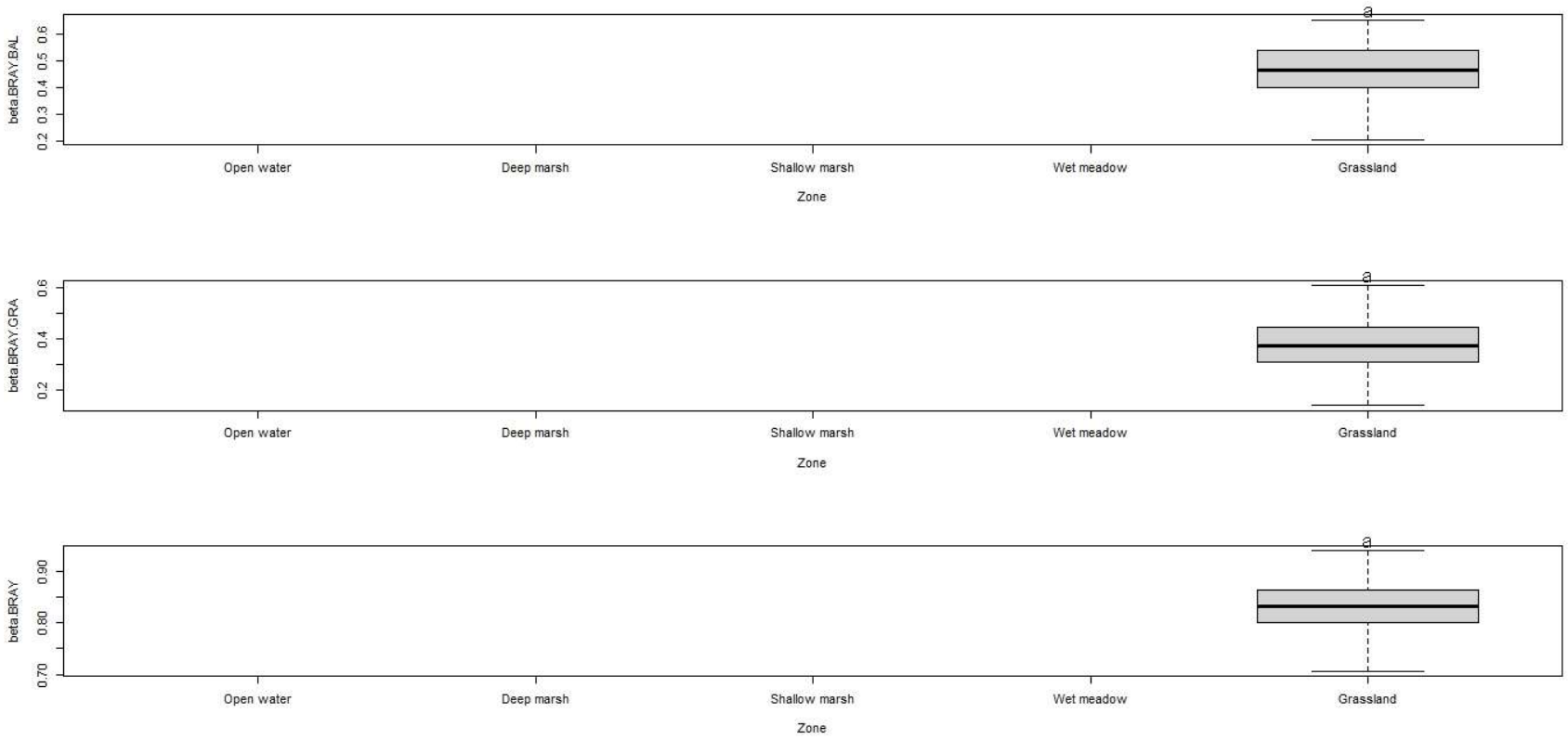
Appendix E. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for dragonflies in the late summer. Different letters above boxes indicate significantly different means.



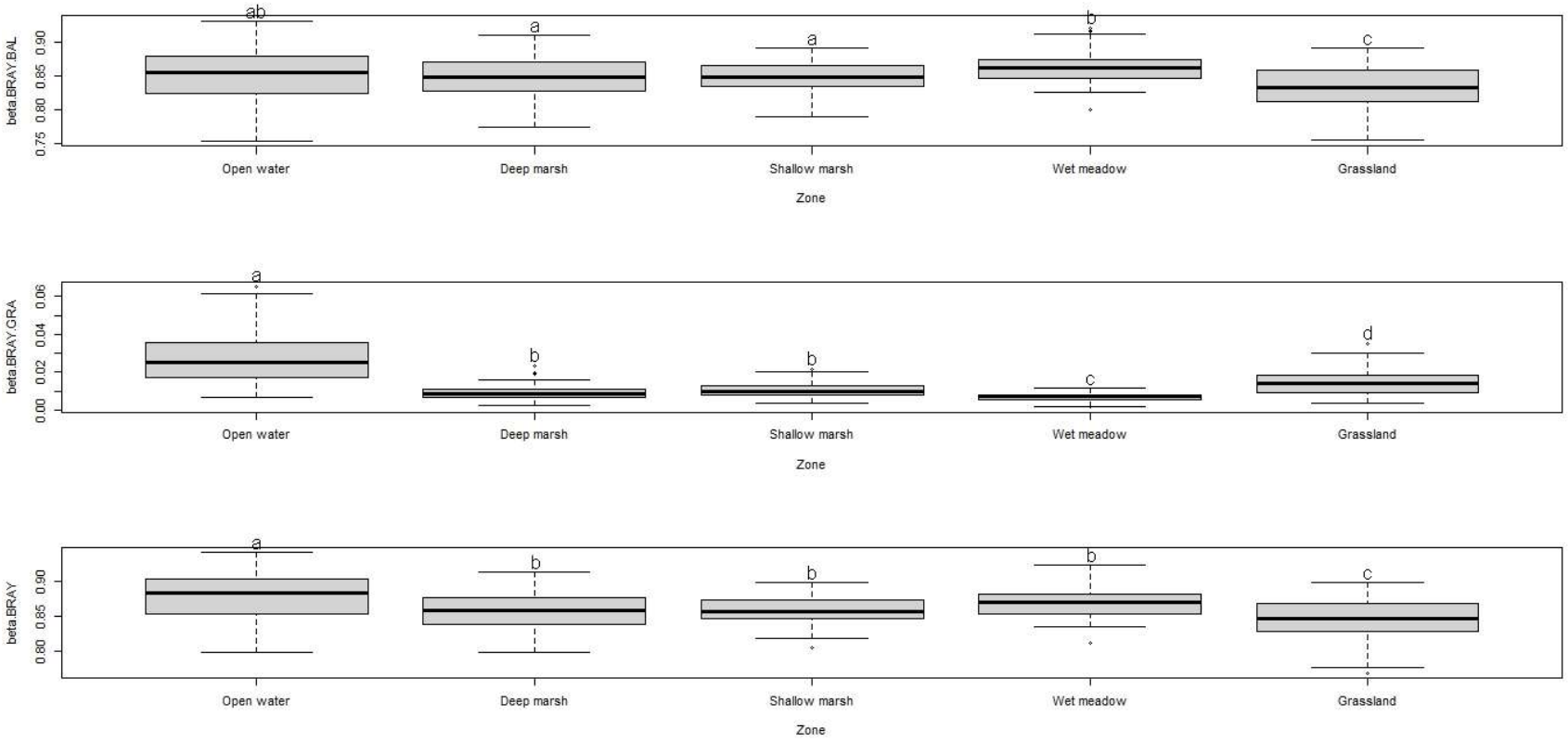
Appendix F. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for butterflies in the early summer. Different letters above boxes indicate significantly different means.



Appendix G. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for butterflies in the late summer. Different letters above boxes indicate significantly different means.



Appendix H. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for grasshoppers in the late summer. Different letters above boxes indicate significantly different means.



Appendix I. Boxplots showing analysis of variance (ANOVA) of beta diversity resampling (100 times) and Tukey post hoc results for plants. Different letters above boxes indicate significantly different means.

Appendix J. Environmental variables measured at each pond.

Site	Plantation	Elevation (m a.s.l.)	Erosion	Water velocity	Soil	Size (m^2)	pH	Water temperature (°C)	Electrical conductivity (µS)	Water depth (cm)	Dissolved oxygen (mg/L)	% plant cover	Plant height (cm)
FH1	Faber's Hill	1508	0	2	clay	1680	7,11	17,66	69,4	28,7	5,31	88	71
FH2	Faber's Hill	1499	0	2	clay	2160	7,5	21,02	76,2	24,7	3,85	87	71
FH3	Faber's Hill	1520	0	1	clay	8550	6,89	19,66	68,7	37,5	3,13	91	100
FH5	Faber's Hill	1411	0	3	sand	2400	7,04	20,25	74,8	12,5	5,13	95	97
FH6	Faber's Hill	1480	0	2	clay	2160	7	20,7	85,9	9,5	3,7	107	94
FH7	Faber's Hill	1467	0	3	sand	2210	7,17	19,97	54,9	7	5,26	108	100
GB1	Gilboa	1445	0	2	clay	2400	7,92	18,5	75,8	62	3,79	85	92
GB2	Gilboa	1471	0	3	clay	2800	6,83	17,31	43,1	34	4,15	115	99
GB3	Gilboa	1523	0	2	clay	7200	6,58	15,37	56,2	13,5	3,11	120	133
GB4	Gilboa	1499	1	1	clay	3000	6,35	18,86	37,3	32	3,07	75	116
GB5	Gilboa	1473	0	1	sand	8500	6,73	16,94	60,4	33,5	2,16	110	76
GB6	Gilboa	1509	0	3	clay	2250	6,22	19,27	53,7	37,5	2,91	110	105
GB7	Gilboa	1483	0	2	sand	5600	6,51	17,68	27,5	44,5	3,2	95	77
GB8	Gilboa	1588	0	1	clay	18000	5,94	22,68	19,5	17	3,51	100	77
GH1	Good Hope	1269	0	2	clay	7500	7,87	22,37	114	30	3,55	85	95
GH2	Good Hope	1345	0	2	clay	8000	7,22	22,04	100,3	20	3,3	115	86
LW1	Linwood	1588	0	2	clay	3750	6,55	20,81	29,3	11,5	2,33	82	55
LW2	Linwood	1150	1	4	sand	2400	7,39	20,89	105	21,5	5,75	80	106
LW3	Linwood	1147	1	4	sand	2200	7,61	25,04	104,9	22	4,97	75	141
LW4	Linwood	962	1	4	sand	2400	8,12	18,75	115	26	5,41	80	125
LW5	Linwood	1013	1	4	sand	2500	8,04	19,58	114,7	26,5	5,15	85	135
MS1	Mount Shannon	1277	0	1	clay	2000	6,93	22,05	104,4	17,5	1,59	93	138

Erosion: 0 – absence, 1 – presence. Water velocity: 1 – still, 2 – slow, 3 – moderate, 4 - fast

Appendix K. Species list: Frogs and invertebrates

Order	Family	Species/morphospecies
Anura	Arthroleptidae	*Leptopelis xenodactylus*
	Bufonidae	*Amietiophrynus gutturalis*
		Amietiophrynus rangeri
		Vandijkophrynus gariepensis
	Hyperoliidae	*Hyperolius semidiscus*
		Kassina senegalensis
		Semnodactylus wealii
	Phrynobatrachidae	*Phrynobatrachus natalensis*
	Pipidae	*Xenopus laevis*
	Ptychadenidae	*Ptychadena anchietae*
		Ptychadena oxyrhynchus
	Pyxicephalidae	*Amietia fuscigula*
		Amietia quecketti
		Cacosternum nanum
		Cacosternum striatum
		Strongylopus fasciatus
		Strongylopus grayii
Odonata (adults)	Aeshnidae	*Anax imperator*
		Anax speratus
		Pinheyschna subpupillata
		Zosteraeschna minuscula
	Calopterygidae	*Phaon iridipennis*
	Coenagrionidae	*Africallagma glaucum*
		Ceriagrion glabrum
		Ischnura senegalensis
		Pseudagrion caffrum
		Pseudagrion hageni
		Pseudagrion kersteni
		Pseudagrion spernatum
	Gomphidae	*Ceratogomphus pictus*
		Notogomphus praetorius
	Lestidae	*Lestes plagiatus*
		Lestes virgatus
	Libellulidae	*Crocothemis erythraea*
		Crocothemis sanguinolenta
		Orthetrum caffrum
		Orthetrum julia falsum
		Palpopleura jucunda
		Sympetrum fonscolombii
		Trithemis dorsalis
		Trithemis furva
		Trithemis stictica
	Platycnemididae	*Allocnemis leucosticta*

		Elattonerua glauca
	Synlestidae	*Chlorolestes fasciatus*
Odonata (larvae)	Aeshnidae	*Anax* sp. 1
		Anax sp. 2
		Zosteraeschna sp.
	Chlorolestidae	*Chlorolestes fasciatus*
	Coenagrionidae	*Agriocnemis* sp.
		Enallagma sp.
		Ischnura sp.
		Pseudagrion sp. 1
		Pseudagrion sp. 2
		Pseudagrion sp. 3
	Gomphidae	*Onychogomphus* sp.
		Paragomphus sp.
	Lestidae	*Lestes* sp.
	Libellulidae	*Orthetrum* sp. 1
		Orthetrum sp. 2
		Trithemis sp. 1
		Trithemis sp. 2
	Platycnemidae	*Platycypha caligata*
Coleoptera	Dytiscidae	*Copelatus* sp. 1
		Copelatus sp. 2
		Hydaticus sp. 1
		Hydaticus sp. 2
		Hydaticus sp. 3
		Hydaticus sp. 4
		Hydrovatus sp.
		Hyphydrus sp.
		Laccophilus sp. 1
		Laccophilus sp. 2
	Elmidae	Sp. 1
	Gyrinidae	*Aulonogyrus* sp.
		Dineutus sp.
		Gyrinus sp.
		Orectogyrus sp.
	Haliplidae	*Haliplus* sp. 1
		Haliplus sp. 2
	Hydraenidae	*Hydraena* sp. 2
		Hydraena sp. 3
		Hydraena sp. 4
		Ochthebius sp. 1
		Ochthebius sp. 2
	Hydrophilidae	*Amphiops* sp. 1
		Amphiops sp. 2
		Berosus sp. 1
		Berosus sp. 2
		Crenitis sp.

		Helochares sp. 1
		Helochares sp. 2
		Hydrophilus sp.
		Laccobius sp.
		Regimbartia sp.
	Noteridae	*Hydrocanthus* sp.
		Neohydrocoptus sp.
	Psephenidae	*Afrobrianax ferdyi*
	Scirtidae	*Elodes* sp.
Hemiptera	Belostomatidae	*Appasus* sp. 1
		Appasus sp. 2
	Corixidae	*Stenocorixa* sp.
	Gerridae	*Eurymetra natalensis*
		Gerris sp.
	Hydrometridae	*Hydrometra* sp.
	Micronectidae	*Micronecta* sp.
	Naucoridae	*Neomacrocoris* sp.
	Nepidae	*Laccotrephes* sp.
		Ranatra sp.
	Notonectidae	*Anisops*
		Notonecta sp.
	Pleidae	*Plea* sp.
	Veliidae	*Microvelia* sp. 1
		Microvelia sp. 2
		Rhagovelia
Lepidoptera	Hesperiidae	*Gegenes pumilio gambica*
		Metisella metis
	Lycaenidae	*Actizera lucida*
		Aloeides aranda
		Cacyreus tespis tespis
		Eicochrysops hippocrates
		Harpendyreus noquasa
		Hypolycaena philippus philippus
		Lampides boeticus
		Lepidochrysops patricia
		Zizeeria knysna
	Nymphalidae	*Aeropetes tulbaghia*
		Cassionympha cassius
		Catacroptera cloanthe cloanthe
		Danaus chrysippus aegyptius
		Hyalites rahira rahira
		Hypolimnas misippus
		Junonia hierta cebrene
		Junonia orithya madagascariensis
		Pardopsis punctatissima
		Pdeudonympha magoides
		Precis octavia natalensis

		Precis octavia sesamus
		Vanessa cardui
	Papilionidae	*Papilio dardanus cenea*
		Papilio nireus lyaeus
	Pieridae	*Belenois aurota aurota*
		Belenois creona severina
		Catopsilia florella
Orthoptera	Acrididae	Sp. 1
		Sp. 2
		Sp. 3
		Sp. 4
		Sp. 5
		Sp. 6
		Sp. 7
		Sp. 8
		Sp. 9
		Sp. 10
		Sp. 11
		Sp. 12
		Sp. 13
		Sp. 14
		Sp. 15
		Sp. 16
		Sp. 17
		Sp. 18
		Sp. 19
		Sp. 20
		Sp. 21
		Sp. 22
		Sp. 23
		Sp. 24
		Sp. 25
		Sp. 26
		Sp. 27
		Sp. 28
		Sp. 29
		Sp. 30
	Lentulidae	Sp. 1
		Sp. 2
		Sp. 3
	Pamphagidae	Sp. 1
	Pyrgomorphidae	Sp. 1
		Sp. 2
	Tetrigidae	Sp. 1
		Sp. 2
	Tettigoniidae	Sp. 1
		Sp. 2
		Sp. 3

	Sp. 4
	Sp. 5
	Sp. 6
	Sp. 7
Tridactylidae	Sp. 1

Appendix L. Species list: Plants

Family	Species/morphospecies
Anthericaceae	*Kniphofia linearifolia*
Apiaceae	*Berula repanda*
Aponogetonaceae	*Aponogeton distachyos*
Araceae	Sp. 1
Araliaceae	*Hydrocotyle americana*
	Sp. 1
Asteraceae	*Cirsium vulgare*
	Conyza bonariensis
	Helichrysum aureonitens
	Helichrysum pilosellum
	Hypochaeris radicata
	Senecio gregatus
	Senecio ilicifolius
	Senecio pterophorus
	Senecio sp.
	Tragopogan dubius
	Sp. 1
	Sp. 2
	Sp. 3
	Sp. 4
	Sp. 5
	Sp. 6
	Sp. 7
	Sp. 8
	Sp. 9
	Sp. 10
	Sp. 11
	Sp. 12
	Sp. 13
	Sp. 14
	Sp. 15
	Sp. 16
	Sp. 17
	Sp. 18
	Sp. 19
	Sp. 20
Brassicaceae	*Nasturtium officinale*
	Sp. 1
Caryophyllaceae	*Pollichia campestris*
Cyperaceae	*Carex austro-africana*
	Cladium mariscus
	Cyperus denudatus
	Cyperus rupestris
	Eleocharis acutangula

	Isolepis fluitans
	Kyllinga alba
	Mariscus congestus
	Pycreus polstachyos
	Schoenoplectus brachyceras
	Sp. 1
	Sp. 2
	Sp. 3
	Sp. 4
Dennstaedtiaceae	*Pteridium aqulinum*
Dryopteridaceae	*Polystichum* sp.
Fabaceae	*Acacia* sp.
	Trifolium repens
	Sp. 1
	Sp. 2
Haloragaceae	*Laurembergia repens*
Illecebraceae	Sp. 1
Iridaceae	*Watsonia lepida*
Juncaceae	*Isolepis prolifera*
	Juncus kraussi
	Juncus lomatophyllus
Lamiaceae	*Mentha aquatica*
	Pycnostachys reticulata
Lemnaceae	*Spirodela* sp.
Malvaceae	*Malva verticillata*
Menyanthaceae	*Nymphoides thunbergiana*
Plantaginaceae	*Plantago major*
Poaceae	*Agrostis montevidensis*
	Aristida sp.
	Cymbopogon caesius
	Ehrharta erecta
	Eragrostis curvula
	Eragrostis plana
	Imperata cylindrica
	Leersia hexandra
	Miscanthus junceus
	Monocymbium ceresiiforme
	Panicum sp.
	Paspalum dilatatum
	Paspalum distichum
	Pennisetum thunbergii
	Phragmites australis
	Setaria sp.
	Setaria sphacelata
	Stiburus alopecuroides
	Themeda triandra
	Sp. 1

	Sp. 2
	Sp. 3
	Sp. 4
	Sp. 5
	Sp. 6
	Sp. 7
	Sp. 8
	Sp. 9
	Sp. 10
	Sp. 11
	Sp. 12
	Sp. 13
	Sp. 14
	Sp. 15
Polygonaceae	*Persicaria decipiens*
	Persicaria lapathifolia
Potamogetonaceae	*Potamogeton pectinatus*
Ranunculaceae	Sp. 1
Rosaceae	*Rubus cuneifolius*
Rubiaceae	*Richardia brasiliensis*
Typhaceae	*Typha capensis*
Verbenaceae	*Verbena bonariensis*